Divergent Antibody Repertoires Found for Omicron versus Wuhan SARS-CoV-2 Strains Using Ig-MS

Eleonora Forte,∥ Benjamin J. Des Soye,∥ Rafael D. Melani,∥ Michael A. R. Hollas, Jared O. Kafader, Beverly E. Sha, Jeffrey R. Schneider, and Neil L. Kelleher*

Cite This: https://doi.org/10.1021/acs.jproteome.2c00514

ABSTRACT: SARS-CoV-2 Omicron (B.1.1.529) and its subvariants are currently the most common variants of concern worldwide, featuring numerous mutations in the spike protein and elsewhere that collectively make Omicron variants more transmissible and more resistant to antibody-mediated neutralization provided by vaccination, previous infections, and monoclonal antibody therapies than their predecessors. We recently reported the creation and characterization of Ig-MS, a new mass spectrometry-based serology platform that can define the repertoire of antibodies against an antigen of interest at single proteoform resolution. Here, we applied Ig-MS to investigate the evolution of plasma antibody repertoires against the receptor-binding domain (RBD) of SARS-CoV-2 in response to the booster shot and natural viral infection. We also assessed the capacity for antibody repertoires generated in response to vaccination and/or infection with the Omicron variant to bind to both Wuhan- and Omicron-RBDs. Our results show that (1) the booster increases antibody titers against both Wuhan- and Omicron-RBDs and elicits an Omicron-specific response and (2) vaccination and infection act synergistically in generating anti-RBD antibody repertoires able to bind both Wuhan- and Omicron-RBDs with variant-specific antibodies.

KEYWORDS: antibodies, SARS-CoV-2, Omicron variant, COVID-19, Ig-MS, serology

INTRODUCTION

COVID-19, which is caused by severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) infection, has affected more than 600 M people and caused the deaths of over 6.5 M people worldwide since late 2019. In response to this virus, the scientific community produced therapeutics and vaccines to protect the population against severe disease and death. Most of the approved or authorized vaccines utilize the viral spike protein, which facilitates the binding of the virus to the host receptor protein angiotensin-converting enzyme 2 (ACE2) via an interaction that is mediated by the spike protein’s receptor-binding domain (RBD). COVID-19 vaccines that use the mRNA platform have a >95% efficacy in preventing severe disease and death by eliciting production of antibodies against spike and RBD that can neutralize, block, or weaken the binding of the spike to ACE2 and inhibit viral entry. Since the emergence of SARS-CoV-2, the virus has evolved to acquire new properties and advantages over a host. A string of variants has emerged featuring mutations conferring increased transmission and immune evasion, enhancing viral propagation and replication. Mutations found in the spike protein, especially in RBD and the N-terminal domain (NTD), are particularly worrisome because they can enable the resulting variants to evade circulating antibodies, thereby reducing the efficacy of vaccines and monoclonal antibody therapeutics, impairing diagnostic tests, and reducing viral neutralization by antibodies produced in response to natural infection. Given the pace of mutation, a method to quickly assess binding and provide robust correlates of protection would help to assess the degree of viral evasion and inform population-level predictions of vaccine effectiveness.

The Delta variant (B.1.617.2), which emerged in India at the end of 2020 and spread worldwide by June 2021, presented a total of nine amino acid mutations, with two in the RBD. In addition to impairing the efficacy of some monoclonal antibodies, those mutations decreased the neutralization efficiency of the available vaccines. However, the vaccines were still able to protect against the Delta variant. On November 26, 2021, a new variant of concern, Omicron (B.1.1.529), was designated by the World Health Organization (WHO). This variant, first identified in South Africa, quickly spread, becoming the dominant SARS-CoV-2 variant in the world. The Omicron variant is highly mutated with more than
30 amino acid changes in the spike protein alone. Many of these mutations are in crucial epitopes of neutralizing antibodies, including the RBD and NTD, and have been shown to confer high resistance to antibody-mediated neutralization. Accordingly, most therapeutic monoclonal antibodies developed against the original SARS-CoV-2 fail to neutralize this variant with the exception of Bebtelovimab, a monoclonal antibody targeting a small conserved region in the RBD, that potently neutralizes Omicron and its subvariants. In addition, antibodies produced in response to natural infection with previous SARS-CoV-2 variants or vaccination have demonstrated reduced neutralization activity against Omicron in pseudovirus neutralization assays.

Several studies performed on vaccinated individuals revealed that two doses of the mRNA vaccine BNT162b2 from Pfizer-BioNTech elicited very low levels of neutralizing antibody against Omicron, resulting in reduced efficacy. However, administering a third booster dose, both homologous and heterologous, potently boosted Omicron neutralization efficiency to the levels previously observed against the original SARS-CoV-2 in individuals who received two doses. This strongly indicates the need for the booster to increase the antibody-mediated protection against the Omicron variant in vaccinated people. Interestingly, people previously infected with an earlier variant of SARS-CoV-2 and then vaccinated have high neutralization against Omicron, suggesting that re-exposure to the antigen stimulates the antibody response to the infection, as expected. Studies on the repertoire of antibodies circulating in the blood of vaccinated individuals that specifically target the Omicron-RBD are crucial to better define the antibody response to this variant.

We have recently developed immunoglobulin-mass spectrometry (Ig-MS), which displays the repertoire of antibodies produced in response to an antigen of interest using protein mass spectrometry. This technology combines an enrichment step (where specific antibodies are isolated using an antigen of interest as bait) with individual ion mass spectrometry (I-MS), an approach capable of determining the exact mass of proteins in highly complex mixtures. Importantly, Ig-MS can determine the relative abundance of the individual proteoforms of antibody clones and calculate the complexity of the repertoire without the need for B cell sequencing. We previously applied Ig-MS to analyze the Ig repertoires responsive to Wuhan-RBD from a cohort of COVID-19 convalescent patients and vaccinated individuals (BNT162b2) and found that antibody titers and degree of clonality were similar for fully vaccinated subjects and patients recovering from severe COVID-19. Here, we apply Ig-MS to (1) analyze how the antibody repertoires raised against Wuhan-RBD in vaccinated individuals change at 6 months post the second dose and after the third dose booster, (2) determine the capability of these repertoires to recognize the Omicron-RBD, and (3) analyze the effect on antibody repertoires in fully vaccinated individuals by subsequent infection with SARS-CoV-2 (Delta and Omicron).

**EXPERIMENTAL SECTION**

**Human Subject Authorizations and Plasma Sampling**

Plasma from fully vaccinated individuals who received two doses of the BNT162b2 vaccine from Pfizer-BioNTech was collected following the NIH guidelines for human subject studies by the clinical team at Rush University Medical Center (IRB NUMBER 00000482) at the following time points: (1) 28 days after the second dose (T1), (2) six months after the second dose (T2), (3) one month after the booster (T3), (4) after infection with Delta SARS-CoV-2, and (5) one month after the booster following infection. In addition, plasma from 6 vaccinated individuals (3 boosted and 3 not boosted) was collected following infection with Omicron SARS-CoV-2 between December 29, 2021, and January 16, 2022 (Table S1). Plasma preparation was conducted as previously reported. As a standard positive control, we used a commercially obtained plasma sample (CS1) from a convalescent patient infected at the beginning of the pandemic (AllCells). As a negative control/blank background, we used pooled serum (Thermo Fisher Scientific, BP2657100 UNSPSC 12352207) collected before the emergence of SARS-CoV-2.

**Anti-Wuhan-RBD Antibody Quantification**

Lumit Dx SARS-CoV-2 Immunoassay Kit (Promega VB1080) was used to determine the relative amounts of antibodies directed against Wuhan-RBD present in plasma from vaccinated and infected subjects following the manufacturer’s instructions. Plasma samples were diluted 1:10 into 1x TBS and heat-inactivated for 1 h at 56 °C prior to running the assay.

**SARS-CoV-2 Spike RBDs Production and Purification**

Wuhan-RBD (amino acids 319–541) was obtained by expressing the plasmid pCAGGS SARS-CoV-2 RBD (BEI Resources, BEI NR-52309) in the Expi293 expression system, followed by purification on ACTAprep (GE Healthcare Life Science) FPLC purification system, as previously reported. B.1.1.529 Omicron-RBD was purchased from Sino Biological (Cat: 40592-V08H121).

**Production of Magnetic Beads Conjugated with Wuhan- or Omicron-RBD**

For this, 0.5 mg of recombinant Wuhan- or Omicron-RBD were covalently bound to 500 μL of Dynabeads MyOne carboxylic acid beads (Thermo Fisher Scientific) following the manufacturer’s instructions. Briefly, the beads were washed twice with 0.5 mL of 25 mM MES, pH 6.0 for 10 min at room temperature and activated by sequentially adding 500 μL of 50 mg/mL N-hydroxysuccinimide (NHS) and 500 μL of mg/mL 1-ethyl-3-(3dimethylaminopropyl) carbodiimide (EDC). After 30 min of incubation at room temperature, the beads were washed twice with 0.5 mL of 25 mM MES, pH 6.0 for 10 min at room temperature and activated by sequentially adding 500 μL of 50 mg/mL N-hydroxysuccinimide (NHS) and 500 μL of mg/mL 1-ethyl-3-(3dimethylaminopropyl) carbodiimide (EDC). After 30 min of incubation at room temperature, the beads were washed twice with 0.5 mL of 25 mM MES, pH 6.0 for 10 min at room temperature and resuspended with 0.5 mL of 25 mM MES, pH 6.0. 0.5 mg of Wuhan- or Omicron-RBD protein were added to the beads and incubated for 30 min at room temperature. Beads were then quenched with 1 mL of 50 mM Tris, pH 6.8, and incubated for 15 min at room temperature. Following four washes with 0.5 mL 1x PBS + 0.1% human serum albumin (HSA), RBD conjugated beads were stored in 1 mL of 1x PBS + 0.1% HSA at 4 °C.

**Enrichment of Anti-RBD Antibodies from Plasma**

The pulldowns were conducted as previously described. In brief, 35 μL of RBD-conjugated beads, 100 μL of plasma, and 865 μL of 1x TBS were combined and incubated overnight at 4 °C with end-over-end mixing. Next, beads were pulled down, and the supernatant discarded. Beads were then resuspended into 1 mL of wash buffer (1x TBS + 0.1% Tween + 1% NP-40 + 1% NP-40 substitute) and transferred onto a KingFisher Flex (Thermo Fisher Scientific). Beads were washed 4 times in 1 mL of wash buffer and twice in 1 mL of 1x TBS. Anti-RBD
antibodies were eluted by incubating the beads for 30 min at 37 °C with 100 μL of 100 mM glycine, pH 11.5.

Preparation of Anti-RBD Light Chains (LC) and Heavy Chains (HC) for Individual Ion Mass Spectrometry

For this, 100 ng of a commercial recombinant mAb were added to each sample as an internal standard (Clone CR3022). LC/HC reduction was conducted by adding 160 μL of 8 M urea and 25 μL of 1 M TCEP to each sample and incubating for 1 h at room temperature. Reduced LCs and HCs were purified by methanol-chloroform-water precipitation and resuspended into 200 μL of 70% water, 30% acetonitrile, and 0.2% formic acid.

Individual Ion Mass Spectrometry (I²MS)

The analysis was performed as previously described. Briefly, samples were delivered to the mass spectrometer at a flow rate of 1 μL/min in a fully automated fashion using a PAL3 robot (CTC Analytics) coupled with a SampleStream flowcell (Integrated Protein Technologies). Samples (70 μL) were sprayed through an Ion Max Source (Thermo Fisher Scientific) fitted with a HESI II probe and analyzed by a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) using the following instrument parameters: eFT, off; trapping gas pressure setting, 0.5; orbitrap central electrode voltage, 1 kV; spray voltage, 2.5−4.0 kV; sheath gas, 0 L/min; in-source CID, 15 eV; source temperature, 320 °C; maximum inject time, 1−400 ms; m/z acquisition range, 650−2000 Th; acquisition time, 60 min; resolving power, 140 000 at 200 m/z.

Data Processing

STORI plot analysis coupled with voting charge assignment algorithm was conducted using StoriBoard (Thermo Fisher Scientific) to determine the charge for each individual ion collected. Briefly, for each ion, we calculated the slope of individual ion signal accumulation as a function of its specific frequency. Individual ion slopes were used to determine the charge for every individual ion signal detected via the StoriBoard voting charge assignment algorithm. Filtered charge assigned individual ions were used to generate I²MS .mzML files.

Figure 1. (A) Schematic illustrating the longitudinal study conducted on four subjects vaccinated with the BNT162b2 mRNA vaccine from Pfizer-BioNTech. Plasma from 4 subjects was collected at 28 days (T1), 6 months (mos.) post the second dose (T2), and at 1 month post the third dose booster (T3). (B) Ig-MS results from representative Subject 1. Spectra of the light chain region are shown for T1 (purple), T2 (dark blue), and T3 (light blue). mAb CR3022, which was used as spike-in standard, is highlighted by a gray rectangle. (C) Ion titer (IT) and (D) antibody diversity (AD) values calculated for all the subjects at all time points are summarized. * P < 0.05; ** P < 0.01; *** P < 0.001.
Calculation of Ig-MS Ion Titer, Degree of Clonality, and Antibody Diversity

Ion titer (IT) and degree of clonality (DoC) values were calculated as previously reported, with an additional noise reduction step added for calculating the DoC. Briefly, we used a custom script to calculate ITs. The .mzML I²MS files were centroided, and spectra were divided into two regions: the LC and the Standard. We calculated the average noise across these regions and subtracted it from the total region intensity. The titers were obtained by determining the ratio between the sum of the standard peak regions and the LC region, divided by the value of the spike-in standard.

DoC, which describes the ratio of the total Ig LC signal vs the total signal of the most abundant clone, was calculated by centroiding profile spectra for a given mass window corresponding to the LC region (excluding the spike-in standard region). For each spectrum, the highest centroid peak was determined, and using an averagine distribution, a window was created around the peak. To account for simple adducts, the window was extended +39 and −18 Da, as determined experimentally. First, noise was determined by calculating the average intensity of centroided peaks in the 21 500–22 000 Da mass range. Second, the noise was multiplied by the signal-to-noise cutoff (3) and subtracted from the intensity of all centroided peaks present in the window created around the highest peak. Third, the noise adjusted intensities in this range were summed and divided by the noise subtracted sum of all intensities in the LC region (excluding the CR3022 standard ranges). Finally, this ratio was subtracted from 1 to yield the DoC:

$$\text{DoC} = 1 - \frac{\sum_{\text{peak window max}} \left(\text{intensity} - \text{noise} \times 3\right)}{\sum_{\text{LC max}} \left(\text{intensity} - \text{noise} \times 3\right)}$$

Antibody diversity (AD) was calculated by centroiding profile spectra of the LC region (22 000–30 000 Da). The centroided peaks corresponding to the CR3022 standard were removed, and the remaining peaks were placed into 1 Da bins. AD was calculated by dividing the number of bins that contain at least one peak above the noise threshold (noise × 3) by the total number of bins.

Data and Statistical Analysis

Unpaired t tests were performed using GraphPad Prism version 9.0.2 for Windows (GraphPad Software) (* P < 0.05; ** P < 0.01; *** P < 0.001) The t-distributed stochastic neighbor embedding (t-SNE) plot was generated using 1 Da bins of the 20 000 to 25 000 Da spectra range on RStudio v1.2.5019 using the Rtsne package and following parameters: 2 dimensions, perplexity of 7, verbose = true, 1000 maximum interactions, and check duplicates = False.

Data and Software Availability

Raw files and processed data sets can be found on the MassiVE repository, MSV000090199. Additional desired software and data that support the findings of this study are available from the corresponding authors upon request.

## RESULTS

### Ig-MS Longitudinal Analysis of Vaccinated Subjects

To characterize the dynamics of the antibody response against Wuhan-RBD over time and in response to a third dose of
BNT162b2 vaccine, we used Ig-MS to analyze the plasma from 4 individuals collected 28 days after the second dose (T1), 6 months after the second dose (T2), and 1 month after the third dose (booster) (T3) (Figure 1A and Table S1). In prior work, we presented data from these same individuals using plasma samples collected before the first vaccine dose (COVID-19 naïve) and at 20 days after the first dose of BNT162b2 (T0)\textsuperscript{15}. Spectra from representative vaccinated Subject 1 are shown in Figure 1B, while those from the other individuals are shown in Figure S1. In agreement with other studies, we found that ion titer (IT) significantly decreased from T1 to T2\textsuperscript{21,22} and rebounded after the booster by an average of 3.5-fold (T3) (Figure 1C). The Lumit immunoassay conducted on the same samples showed the same trends (Figure S2). We used two distinct metrics for assessing the complexity of the antibody response, the previously described degree of clonality (DoC)\textsuperscript{15} and a new metric presented here named antibody diversity (AD), which quantifies peak presence in the LC region. AD decreased from T1 to T2 followed by a slight increase postbooster (T3) (Figures 1D and S2). For example, for subject 1, the observed AD values varied from 21.6 in T1 to 0.19 in T2 and 5.03 in T3. The spectra clearly showed that the number of different antibody clones at T1 is much higher than T3. Interestingly, the latter collection point features 4 main peaks also observed at T1, though many clones detectable at T1 are no longer visible at T3. We did not observe significant changes between the three conditions for DoC (Figure S2).

To determine whether and to what extent the binding capabilities of antibody repertoires elicited in response to Wuhan-RBD were affected by the mutations present in the Omicron-RBD, we next performed Ig-MS assays on the plasma...
samples collected at times T2 and T3 using the Omicron-RBD as bait. Omicron-RBD was commercially obtained and conjugated to beads following the same protocol used for the Wuhan-RBD. Anti-Wuhan- and Omicron-RBD antibodies were then enriched, reduced, and analyzed by SampleStream and I²MS. Spectra derived from the representative Subject 4 are shown in Figure 2A and B, respectively. The spectra from the other individuals are shown in Figure S1.

Overall, our analysis revealed a modest capacity for antibody repertoires produced in response to Wuhan-RBD to recognize and bind to Omicron-RBD. Observed IT trends were very similar using both RBD variants as bait, increasing in all the subjects by an average of ∼3.5- and 2.6-fold from T2 to T3 for Wuhan and Omicron, respectively. However, absolute IT values were ∼20−70% higher in assays using Wuhan-RBD compared to those using Omicron-RBD (Figure 2C). Repertoire complexities were observed to be very similar regardless of the bait protein used, with AD increasing between T2 and T3 for both RBDs (Figure 2D). No differences in DoC were observed (Figure S2). These results suggest that (1) at 6 months post second dose (T2), the amount of antibodies circulating in the plasma directed against Wuhan-RBD is generally low, and the titer of antibodies capable of recognizing and binding Omicron-RBD is not significantly different, (2) the booster (T3) increases the IT values for antibodies directed against both Wuhan- and Omicron-RBDs, however, the titer of antibodies able to bind Omicron-RBD is significantly lower than those able to bind Wuhan-RBD, and (3) the repertoire complexity increases after the booster regardless of the kind of RBD used as bait.

**Ig-MS Analysis of Infected Subjects**

We next applied Ig-MS using both RBD variants as bait to two fully vaccinated subjects infected with SARS-CoV-2 seven months after receiving the second vaccine dose. At the time of infection, the Delta variant (B.1.617.2) was the dominant circulating strain in the collection region. Notably, one of the infected subjects received the booster one month after infection (Subject 6), while the other did not (Subject 5) (Figure 3A). As a control, we first analyzed plasma from CS1, an unvaccinated convalescent COVID-19 patient, that was commercially obtained (Figure 3B,E). We found that the repertoires of antibodies able to recognize Wuhan-RBD had an IT almost 3-times higher than that for Omicron-RBD (8.8 and 2.6, respectively). In addition, the AD value observed using the Omicron Ig-MS assay was significantly lower than that obtained with the Wuhan-RBD Ig-MS assay (4.7 and 11.3, respectively). The spectra clearly demonstrate that many peaks corresponding to antibody clones that recognize Wuhan-RBD are absent from corresponding Omicron Ig-MS spectra, suggesting that those clones do not recognize the Omicron-RBD. A striking example is the main peak at ∼22.6 kDa in the Wuhan-RBD spectrum that is completely missing from the Omicron-RBD spectrum (Figure 3B,E). The analysis of the plasma from Subject 5 showed a lower titer of antibodies able to bind Omicron-RBD compared to Wuhan-RBD, with calculated IT values of 5.3 and 8.7, respectively (Figure 3C,F). Interestingly, the observed complexity of the antibody repertoire binding the Omicron-RBD was slightly higher than that for Wuhan-RBD, with respective AD values of 10.8 and 7.7 (Figure 3C,F). In particular, the spectrum of antibody LC clones that recognize Wuhan-RBD is dominated by four main...
peaks, some of which are lost in the corresponding Omicron-RBD spectrum, though the latter spectrum features a higher number of low-abundance species. Analysis of samples from Subject 6 revealed a similar trend (Figure 3D,G). We observed IT values of 8.9 and 6.4 for the antibody repertoire recognizing Wuhan-RBD and Omicron-RBD, respectively. Also, consistent with what was observed in Subject 5, the antibody repertoire binding the Omicron-RBD shows higher diversity than the Wuhan-RBD, with AD values of 15 and 9.3, respectively. The spectra suggest that antibody repertoires generated in response to immune challenge with antigens originating from non-Omicron variants of SARS-CoV-2 contain some clones that can also recognize and bind to Omicron-derived epitopes.

From the analysis of this small cohort, our results suggest that infection is synergistic with vaccination, generating a higher number of antibodies that can bind Omicron-RBD compared to vaccination alone. In this case, the booster 1 month post-infection seems to slightly improve circulating antibody repertoires’ capabilities to bind to Omicron-RBD both in terms of IT and AD. Of course, these are observations made on only two subjects. Further studies using larger cohorts will have more significance.

Finally, we performed the Ig-MS assay on plasma samples collected from individuals infected specifically with the Omicron variant using both Wuhan and Omicron spike RBDs as bait. The cohort comprised plasma samples from 3 subjects who received the second vaccine dose 8 and 10 months before the infection (Subjects 7, 8, and 11) as well as plasma from 3 subjects who got the booster 3 to 5 months before the infection (Subjects 9, 10, and 12). For each subject, plasma was collected between 6 and 29 days after the breakthrough infection. Spectra of LC repertoires from representative subjects for each condition are shown in Figure 4A (Subject 7) and B (Subject 10), while all other subjects are presented in Figure S3.

In all subjects (regardless of boosted status), the repertoires of antibodies able to bind Wuhan- or Omicron-RBD were observed to differ, as evident from the distribution and the intensities of peaks in the spectra. Some of the peaks observed were unique for each RBD, while others were present in spectra for both RBDs at different intensities. We observed changes in the antibody response from subject to subject, both in terms of titers and diversity. Overall, the data demonstrated no significant differences in the average IT or AD values (Figure 4C,D) determined for antibody repertoires binding either RBD from individuals with or without the booster. This could be due to differences within the subject population and limited cohort size. However, when the subjects were evaluated individually, antibody repertoires that bind the Omicron-RBD had overall higher AD than those binding the Wuhan-RBD, suggesting that there are more distinct antibody clones in circulation capable of binding the Omicron-RBD after infection with the variant (Figure 4E).

Interestingly, the DoC values observed in anti-Omicron-RBD antibody repertoires were generally lower in the subset of patients that received the booster compared to the anti-Wuhan-RBD antibody repertoires. This indicates that even though there are more clones that recognize Omicron-RBD in these samples, the response is dominated by a small number of highly abundant clones (Figures S2 and S3). In addition, the DoC values of antibody repertoires binding to Omicron-RBD were generally lower in subjects that received the booster as compared to those that did not, a result that we attribute to affinity maturation triggered by the booster (Figure S2).

To summarize all the data collected throughout this study, we generated a t-SNE plot representing the antibody responses against Wuhan- and Omicron-RBDs of all the analyzed vaccinated and/or infected subjects by using the mass intensity values between 20 000 and 25 000 Da obtained from the Ig-MS analyses. Figure 5 shows well-defined clusters for all different groups of samples, with the only exception being the sample from Subject 4 collected at T3. Specifically, following the third booster dose, this subject showed a limited number of antibodies able to bind the Omicron-RBD (Figure S1). Consequently, this sample clustered together with samples collected at T2. The t-SNE plot showed that (1) samples collected at T1 had the highest immune response and clustered on the left side of the plot; (2) samples collected at T2 had less abundant and diverse LC repertoires and clustered together on the opposite side independently of the RBD used for the Ig-MS analysis; and (3) samples from vaccinated subjects collected at T3 and vaccinated individuals infected with the
Delta or Omicron variants cluster together in the middle of the plot, suggesting that the immune response in boosted individuals is similar to convalescent subjects, confirming the importance of the third dose. Additionally, LC repertoires against Wuhan-RBD from samples collected at T3, which have significantly higher ITs than those against Omicron-RBD, cluster close to those collected at T1. The same trend is observed for LC repertoires against Omicron-RBD from subjects infected with Omicron.

**DISCUSSION**

**Feasibility of Ig-MS to Inform Vaccination Deployment**

In response to the COVID-19 pandemic, several vaccines have been authorized for use to suppress SARS-CoV-2 infection, transmission, and prevent severe disease. The mRNA vaccines developed by Moderna (mRNA-1273) and Pfizer-BioNTech (BNT162b2) trigger an immune response against the viral spike protein, a main target of neutralizing antibodies due to its strategic function in facilitating viral entry into cells. These vaccines showed an efficacy close to 100% against severe illness and death in the first clinical trials, however, longitudinal studies conducted after the second dose indicated that the vaccine’s protection diminished over time with concomitant decreases in antibody titers and neutralization activity over a 5–6 month period. A third dose was able to boost antibody titer and neutralization, making an additional dose critical for increasing the protection against severe disease. This study highlights the modularity and facile adaptability of Ig-MS to different antigens, as changing the workflow to enrich for antibodies specifically against the Omicron variant RBD sequence was as straightforward as exchanging this variant RBD for the Wuhan-RBD in the bead fabrication protocol. Therefore, Ig-MS could be capable of being deployed on a time scale to inform vaccination policies for any pathogen of interest. In the future, more subjects will be required to determine if the new data type produced by Ig-MS could serve as a reliable “correlate of protection” without the need for functional studies like neutralization assays.

**Ig-MS Using Wuhan-RBD across Time**

We focused on antibodies against the RBD portion of the spike because of its key function in binding the host receptor and high immunogenicity. After the second and third dose of BNT162b2 in 4 subjects, we found that the titer of total anti-RBD antibodies was extremely low at 6 months after the second dose with very few clones above the limit of detection and that titers recovered after administration of a third booster. Neutralization and ELISA assays performed both by us and others similarly demonstrated the ability of the booster to strongly increase this antibody titer. Furthermore, it was shown that the titers of antibodies observed 1 month post booster were similar to or higher than those present 1 month post the second dose. In our analysis, there was not a significant difference between these two collection points when we calculated the titers with the Lumit test. However, Ig-MS analysis suggested that the IT titers were lower after the third dose. This could be due to intrinsic differences in the two assays. In terms of the antibody diversity, we observed a significantly higher number of individual clones between 6 months post second dose and 1 month post booster. However, these are significantly lower than those present at 1 month after the second dose, with a repertoire mainly consisting of a few persistent abundant clones, likely due to affinity maturation and clone selection.

In addition to waning levels of circulating antibodies, vaccine efficacies are also challenged by the emergence of new SARS-CoV-2 variants, such as the Omicron variant, which feature a very high number of mutations that collectively reduce (and in some cases abolish) the neutralization potential of the majority of monoclonal antibodies targeting RBD, including most approved for clinical use and those elicited by previous infection and vaccination. To investigate this differential capacity for patient antibody repertoires to recognize and bind Wuhan- vs Omicron-RBD, we performed Ig-MS on the vaccinated subject samples using both RBDs individually as the bait antigen. We found that after 6 months post the second dose, there were no significant differences between the two repertoires in terms of titer and diversity. The booster increased the IT values for antibodies directed against both Wuhan and Omicron; however, the level of antibodies able to bind Omicron-RBD was significantly reduced compared to that able to bind Wuhan-RBD. This is consistent with previous reporting. In addition, the repertoire complexity increases after the booster, regardless of the kind of RBD used as bait. These results indicate that the booster produces antibodies that recognize and bind the variant RBD including some that exclusively bind to Omicron-RBD (though these are at significantly lower levels than those that bind Wuhan-RBD). Interestingly, while the titers of the antibodies recognizing Omicron-RBD were lower than to those binding the Wuhan-RBD, the number of different clones present in the spectra was comparable between the two RBDs.

**Wuhan-RBD vs Omicron-RBD**

We also conducted Ig-MS using both Wuhan-RBD and Omicron-RBD bait antigens on samples derived from subjects who became infected with SARS-CoV-2 after receiving two doses of vaccine. It is worth highlighting that the subjects used for this series of experiments got infected prior to the emergence of Omicron so they specifically did not get infected with this variant. A control experiment conducted on the serum of a COVID-19 convalescent patient infected at the early stage of the pandemic (CS1) indicated that antibodies produced against Wuhan-RBD mostly fail to recognize the Omicron-RBD. However, the titers of antibodies recognizing the Omicron-RBD were higher in samples derived from individuals infected after receiving two vaccine doses, suggesting a synergistic effect of immunity acquired by both natural infection and vaccination, which has been described previously.

Finally, we applied Ig-MS to analyze the response to both Wuhan- and Omicron-RBD in subjects with confirmed Omicron variant infections after receiving 2 or 3 vaccine doses. The comparison between the repertoires recognizing the Wuhan- and Omicron-RBDs indicated the presence of clones that recognize only one of the two RBDs and others that can bind both RBDs but in different relative amounts. Overall, no significant differences between the two repertoires were observed in terms of total titer and number of clones and between boosted and nonboosted subjects. These results are consistent with animal studies that show similar immunity using either the original mRNA vaccine booster or a Omicron-specific mRNA booster. However, the analysis of the individual subjects indicates that both titer and the absolute number of clones generally increase in the repertoires.
recognizing the Omicron-RBD, especially in boosted subjects. In addition, in the boosted subjects, there is a low number of prevalent clones binding to the Omicron-RBD that stand out over several lower intensity clones that are selected and expanded by affinity maturation driven by a fourth exposure to the antigen (3 vaccine doses and an infection).

**CONCLUSION**

This study provides a glimpse of the evolution of the antibody response over time for individuals vaccinated and infected at single clone resolution and strongly indicates that the booster is essential for obtaining higher antibody protection against the SARS-CoV-2 Omicron variant, even in subjects that have been previously infected by other variants. Future studies on antibodies targeting non-RBD spike epitopes should also be included. Indeed, it was reported that, although the RBD region is the epitope receiving the most attention, the antibody response targets many viral antigens outside the RBD, with many neutralizing antibodies targeting the spike N-terminal domain that is also commonly mutated in the variants causing COVID-19.  

**ASSOCIATED CONTENT**

**Data Availability Statement**

Raw files and processed data sets can be found on the MassiVE repository, MSV000090199. Additional desired software and data that support the findings of this study are available from the corresponding authors upon request.

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.2c00514.

**Figure S1.** LC spectral region from 4 subjects at time points T2 and T3 obtained by Ig-MS with Wuhan Spike-RBD or Omicron Spike-RBD. **Figure S2.** Anti-Wuhan RBD antibody titers calculated with a bioluminescent in vitro diagnostic and DoC values of the entire cohort. **Figure S3.** LC spectral regions from 3 non-boosted and 3 boosted subjects obtained by Ig-MS with Wuhan Spike-RBD or Omicron Spike-RBD. **Table S1.** Demographics and dates of vaccination, infection, and plasma collection (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**

Neil L. Kelleher — Proteomics Center of Excellence, Evanston, Illinois 60208, United States; Department of Surgery, Comprehensive Transplant Center, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, United States; Department of Biochemistry and Molecular Genetics, Northwestern University Feinberg School of Medicine, Chicago, Illinois 60611, United States; Email: n-kelleher@northwestern.edu

**Authors**

Eleonora Forte — Proteomics Center of Excellence, Evanston, Illinois 60208, United States; Department of Surgery, Comprehensive Transplant Center, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, United States

Benjamin J. Des Soye — Proteomics Center of Excellence, Evanston, Illinois 60208, United States; orcid.org/0000-0001-5480-4192

Rafael D. Melani — Proteomics Center of Excellence, Evanston, Illinois 60208, United States; Department of Molecular Biosciences, Chemistry, Northwestern University, Evanston, Illinois 60208, United States

Michael A. R. Hollas — Proteomics Center of Excellence, Evanston, Illinois 60208, United States; orcid.org/0000-0002-0797-3134

Jared O. Kafader — Proteomics Center of Excellence, Evanston, Illinois 60208, United States; orcid.org/0000-0002-5359-264X

Beverly E. Sha — Division of Infectious Diseases, Rush University Medical Center, Chicago, Illinois 60612, United States; orcid.org/0000-0002-9659-3554

Jeffrey R. Schneider — Department of Microbial Pathogens and Immunity, Rush University Medical Center, Chicago, Illinois 60612, United States

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jproteome.2c00514

**Author Contributions**

©F.E., B.J.D., and R.D.M. contributed equally to this work.

**Notes**

The authors declare the following competing financial interest(s): Neil Kelleher and Jared Kafader report a conflict of interest with FMS technology, which Thermo Fisher Scientific is commercializing as Direct Mass Technology. The other authors declare no conflicts of interest.

**ACKNOWLEDGMENTS**

Wuhan-RBD was produced by the Northwestern Recombinant Protein Production Core Facility. The reagent was produced under HHSN272201400008C and obtained through BEI Resources, NIAID, NIH: Vector pCAGGS Containing the SARS-Related Coronavirus 2, Wuhan-Hu-1 spike Glycoprotein Receptor-Binding Domain (RBD), NR-52309. This study was supported by the National Institute of Health, specifically by the National Institute of General Medical Sciences P41 GM108569 (N.L.K.). However, the content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Additional funding was received from the Research Corporation (Grant No. 27372, N.L.K. and E.F.). This work was also supported by the Walder Foundation’s Chicago Coronavirus Assessment Network (Chicago CAN) Initiative Grant Nos. SC116, 21-00147, and 21-00650 (J.R.S.).

**REFERENCES**

(1) (a) Patel, R.; Kaki, M.; Potluri, V. S.; Kahar, P.; Khanna, D. A comprehensive review of SARS-CoV2 vaccines: Pfizer, Moderna & Johnson & Johnson. *Hum Vaccin Immunother* 2022, 18 (1), No. 2002083. (b) Kumar, S.; Caliskan, D. M.; Janowski, J.; Faist, A.; Conrad, B. C. G.; Lange, J.; Ludwig, S.; Brunotte, L. Beyond Vaccines: Clinical Status of Prospective COVID-19 Therapeutics. *Front Immunol* 2021, 12, No. 752227. (c) (d) Du, L.; Yang, Y.; Zhang, X. Neutralizing antibodies for the prevention and treatment of COVID-19. *Cell Mol. Immunol* 2021, 18 (10), 2293–2306.

(2) (a) Walls, A. C.; Park, Y. J.; Tortorici, M. A.; Wall, A.; McGuire, A. T.; Veesler, D. Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell 2020*, 181 (2), 281–292. (b) Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q;
Zhang, L.; et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020, 581 (7807), 215–220.

(3) Mulligan, M. J.; Lyke, K. E.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Neuzil, K.; Raabe, V.; Bailey, R.; Swanson, K. A.; et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature 2020, 586 (7830), 589–593.

(4) Jackson, L. A.; Anderson, E. J.; Rouphael, N. G.; Widge, A. T.; Jackson, L. A.; Roberts, P. C.; Makhene, M.; Chappell, J. D.; Denison, M. R.; Stevens, L. J.; et al. Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. N Engl J Med 2020, 383 (25), 2427–2438.

(5) Hastie, K. M.; Li, H.; Bedinger, D. S.; Schendel, S. L.; Dennison, S. M.; Li, K.; Rayment, A.; Yu, X.; Mann, C.; Zandonatti, M.; et al. Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: A global consortium study. Science 2021, 374 (6566), 472–478.

(6) Abu-Raddad, L. J.; Chemaitelly, H.; Butt, A. A. Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. N Engl J Med 2021, 385 (2), 187–189.

(7) Lopez Bernal, J.; Andrews, N.; Gower, C.; Gallagher, E.; Simmons, R.; Thellwall, S.; Stowe, J.; Tessier, E.; Groves, N.; Dabrera, G.; et al. Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. N Engl J Med 2021, 385 (7), 585–594.

(8) Garcia-Beltran, W. F.; Lam, E. C.; St John, S. M.; Mok, C. K. P.; Leung, Y. W. Y.; Ng, S. S.; Chan, K. C. K.; Ko, F. S.; Chen, C.; Yu, K.; Lam, B. H. S.; Lai, E. H. Y.; et al. Neutralizing antibodies against the SARS-CoV-2 Omicron variant BA.1 following homologous and heterologous CoronaVac or BNT162b2 vaccination. Nat Med 2022, 28, 486.

(9) Schmidt, F.; Muecksch, F.; Weißen, D.; Da Silva, J.; Bednarcki, E.; Cho, A.; Wang, Z.; Gaebler, C.; Caskey, M.; Nussenreng, M. C.; et al. Plasma Neutralization of the SARS-CoV-2 Omicron Variant. N Engl J Med 2022, 386 (6), 599–601.

(10) Kafader, J. O.; Beu, S. C.; Fellers, R. T.; Beu, S. C.; Mazokov, A. A.; Maze, T. J.; et al. Multiplexed mass spectrometry of individual ions improves measurement of proteoforms and their complexes. Nat Methods 2022, 19 (4), 391–394.

(11) Toby, T. K.; Fornelli, L.; Szarcinski, K.; DeHart, C. J.; Levitsky, J.; Friedewald, J.; Kelleher, N. L. A comprehensive pipeline for translational top-down proteomics from a single blood draw. Nat Protoc 2019, 14 (1), 119–152.

(12) Skinner, O. S.; Havugimana, P. C.; Haverind, N. A.; Fornelli, L.; Early, B. P.; Greer, J. B.; Fellers, R. T.; Durbin, K. R.; Do Yale, L. H.; Melani, R. D.; et al. An informatic framework for decoding protein complexes by top-down mass spectrometry. Nat Methods 2016, 13 (3), 237–240.
spectra of large molecules. J. Am. Soc. Mass Spectrom. 2000, 11 (4), 320–332.

(21) Nauber, P.; Tserel, L.; Kangro, K.; Sepp, E.; Jurjenson, V.; Adamson, A.; Haljasmagi, L.; Rumm, A. P.; Maruste, R.; Karner, J.; et al. Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. Lancet Reg Health Eur. 2021, 10, No. 100208.

(22) Levin, E. G.; Lustig, Y.; Cohen, C.; Fluss, R.; Indenbaum, V.; Amit, S.; Doolman, R.; Asraf, K.; Mendelson, E.; Ziv, A.; et al. Waning Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. N Engl J. Med. 2021, 385 (24), e84.

(23) Baden, L. R.; El Sahly, H. M.; Essink, B.; Kotloff, K.; Frey, S.; Novak, R.; Diemert, D.; Spector, S. A.; Roupheal, N.; Creech, C. B.; et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J. Med. 2021, 384 (5), 403–416.

(24) (a) Thomas, S. J.; Moreira, E. D., Jr.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J. L.; Perez Marc, G.; Polack, F. P.; Zerbini, C.; et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. N Engl J. Med. 2021, 385 (19), 1761–1773. (b) Doria-Rose, N.; Suthar, M. S.; Makowski, M.; O’Connell, S.; McDermott, A. B.; Flach, B.; Ledgerwood, J. E.; Mascola, J. R.; Graham, B. S.; Lin, B. C.; et al. Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid-19. N Engl J. Med. 2021, 384 (23), 2259–2261.

(25) Pegu, A.; O’Connell, S. E.; Schmidt, S. D.; O’Dell, S.; Talana, C. A.; Lai, L.; Albert, J.; Anderson, E.; Bennett, H.; Corbett, K. S.; et al. Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants. Science 2021, 373 (6561), 1372–1377.

(26) Falsey, A. R.; Frenck, R. W., Jr.; Walsh, E. E.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Bailey, R.; SwanSON, K. A.; Xu, X.; et al. SARS-CoV-2 Neutralization with BNT162b2 Vaccine Dose 3. N Engl J. Med. 2021, 385 (17), 1627–1629.

(27) Chu, L.; Vrbbicky, K.; Montefiori, D.; Huang, W.; Nestorova, B.; Chang, Y.; Carfi, A.; Edwards, D. K.; Oestreich, J.; Legault, H.; et al. Immune response to SARS-CoV-2 after a booster of mRNA-1273: an open-label phase 2 trial. Nat. Med. 2022, 28, 1042.

(28) Planas, D.; Saunders, N.; Maes, P.; Guível-Benhassine, F.; Planchais, C.; Buchrieser, J.; Bolland, W. H.; Porrot, F.; Staropoli, L.; Lemoine, F.; et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 2022, 602 (7898), 671–675.

(29) Premkumar, I.; Segovia-ChumbeB, B.; Jadi, R.; Martinez, D. R.; Raut, R.; Markmann, A.; Cornaby, C.; Bartelt, L.; Weiss, S.; Park, Y. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. Sci. Immunol. 2020, 5 (48), 1 DOI: 10.1126/sciimmunol.abc8413.

(30) Choi, A.; Koch, M.; Wu, K.; Chu, L.; Ma, L.; Hill, A.; Nunna, N.; Huang, W.; Oestreich, J.; Colpitts, T.; et al. Safety and immunogenicity of SARS-CoV-2 variant mRNA vaccine boosters in healthy adults: an interim analysis. Nat. Med. 2021, 27 (11), 2025–2031.

(31) (a) Cao, Y.; Wang, J.; Jian, F.; Xiao, T.; Song, W.; Yisimayi, A.; Huang, W.; Li, Q.; Wang, P.; An, R.; et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. Nature 2022, 602 (7898), 657–663. (b) Liu, L.; Iketani, S.; Guo, Y.; Chan, J.; F.; Wang, M.; Liu, L.; Luo, Y.; Chu, H.; Huang, Y.; Nair, M. S.; et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. Nature 2022, 602 (7898), 676–681.

(32) (a) Hein, S.; Mhedhbi, I.; Zahn, T.; Sabino, C.; Benz, N. I.; Husria, Y.; Renelt, P. M.; Braun, F.; Oberle, D.; Maier, T. J. Quantitative and Qualitative Difference in Antibody Response against Omicron and Ancestral SARS-CoV-2 after Third and Fourth Vaccination. Vaccines (Basel) 2022, 10 (5), 796. (b) Zuo, F.; Abolhasann, H.; Du, L.; Piralla, A.; Bertoglio, F.; de Campos-Mata, L.; Wan, H.; Schubert, M.; Cassantini, I.; Wang, Y.; et al. Heterologous immunization with inactivated vaccine followed by mRNA-booster elicits strong immunity against SARS-CoV-2 Omicron variant. Nat. Commun. 2022, 13 (1), 2670.