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Humoral Responses to SARS-CoV-2 Infection and mRNA Vaccination

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Humoral Responses to SARS-CoV-2 Infection and mRNA Vaccination

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Abstract

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First detected in 2019, SARS-CoV-2 remains a global pandemic with over 430 million individuals infected to date. Significant homology between SARS-CoV-2 and -1 allowed the rapid identification of the spike (S) protein, specifically the receptor binding domain (RBD), as a critical target for neutralizing antibodies. Given the rapid spread of the virus, characterizing potentially protective aspects of the immune response to infection was urgent. In a cohort of individuals hospitalized with severe COVID-19, we observed robust B cell responses, detecting expanded plasmablasts, activated RBD-specific memory B cells, and elevated titers of RBD- and S-specific antibodies. Depletion of RBD-specific antibodies from serum significantly reduced neutralizing activity in the majority of individuals. However, some donors retained significant residual neutralization activity, suggesting a potentially protective antibody subset targeting non-RBD epitopes. This study demonstrated that SARS-CoV-2 infection induces a robust humoral response and that RBD-specific antibodies are critical for circulating viral neutralization in infected individuals.

Soon after the emergence of SARS-CoV-2, hundreds of vaccines went into pre-clinical and clinical testing in an effort to combat the growing global pandemic. By the end of 2020, the U.S. had approved two mRNA-based vaccines from Moderna and Pfizer. While initial reports of efficacy were high for both vaccines, the emergence of several SARS-CoV-2 variants raised questions about the breadth and durability of the vaccine-induced humoral response. To this end, we analyzed the humoral response to either Moderna or Pfizer mRNA vaccination in a longitudinal cohort of naïve and recovered individuals. We found that, while vaccination induced SARS-CoV-2 specific humoral immunity in both groups, the antibody response was both more robust and durable in recovered individuals than naïve individuals and that vaccine responses positively correlated with initial responses to infection in recovered individuals. Despite the similarity of the mRNA vaccines, Moderna-vaccinated naïve individuals demonstrated a less reliance on RBD-specific antibodies for neutralization than Pfizer vaccinees. Taken together, these studies illustrate that both infection and vaccination against SARS-CoV-2 can elicit potent and protective humoral responses.

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CHAPTER 1

INTRODUCTION

B CELLS IN VIRAL INFECTIONS

When an individual is exposed to a viral pathogen, a carefully orchestrated immune response occurs involving both innate and adaptive immune cells. B cells are integral to this response, primarily due to their role as the sole producers of antibodies, secretory proteins that have the ability to inhibit the viral life cycle through a variety of pathways (1, 2). When viral particles traffic to lymphatic structures in acute infection, their interaction with antigen-specific receptors present on B cells (BCRs) leads to activation of naïve B cells and can induce both differentiation and receptor affinity maturation (1, 2). Plasmablasts, short-lived B cells that transition to actively secreting their BCR, now known as antibody, are normally considered the first B cell subset to expand in infection (1, 2). As the infection progresses, activated B cells can enter structures in the lymphatics known as germinal centers, where B cells undergo processes of affinity maturation including somatic hypermutation and class-switching (1, 2). Germinal center B cells can then differentiate to become either memory B cells (MBCs), which can be quickly reactivated upon re-exposure to the pathogen, or plasma cells, long lived antibody secreting cells that primarily traffic to the bone marrow compartment (1, 2). In this way, a single viral exposure can prime a recall immune response that can quickly respond to re-infection, often years later. The establishment of this B cell response is the basis for the majority of vaccine strategies employed today.

B Cell Activation

B cells are a major component of the adaptive immune response and can be quickly activated through either T-cell independent or dependent pathways in the context of viral infections. A subset of B cells can be activated by either strong signaling through innate receptors or multivalent antigen engagement of their BCR (2). Through this pathway, B cells can

be activated simply through interaction with whole, unprocessed antigen trafficked into the lymph node during infection. However, T-cell dependent B cell activation requires not only signaling through the BCR but the engagement of various co-stimulatory receptors on helper T cells, including the binding of the cognate T cell receptor with MHC-presented peptide on the B cell (2). Evidence from animal models has additionally demonstrated that the early interferon responses present in viral infection aid in lowering the threshold for B cell activation through the upregulation of these co-stimulatory markers (3, 4) and that mice lacking the IFN receptor have defective B cell responses in the context of influenza infection (5).

Once activated, B cells can differentiate into effector subsets in the extrafollicular space or migrate to germinal centers for further affinity maturation of their antigen receptors. The signals directing the recruitment of B cells to each of these paths are still under investigation, but current evidence suggests that BCR affinity plays a role in the differentiation (6, 7) and expansion (8) of B cell effector subsets with B cells with higher affinity receptors participating in the immediate humoral response and lower affinity B cells trafficking to the germinal center. Each of these pathways are traditionally thought to control distinct processes of B cell differentiation and maturation, but studies conducted within the last 20 years have illustrated that many processes thought to be restricted to the germinal center can actually occur within the extrafollicular space.

Extrafollicular Responses

While initially thought to only generate a pool of effector cells with low-affinity to antigen, recent studies have determined that extrafollicular responses can generate B cells that have undergone both somatic hypermutation (9) and class switch recombination (10, 11). In fact, mice unable to properly form germinal centers are found to be still able to produce high-affinity

antibody responses (12), suggesting that extrafollicular responses play a more complex role in the long-term B cell response to infection. The extrafollicular response occurs rapidly after infection and is thought to be the primary source of plasmablasts, a short-lived antibody-secreting B cell subset. Plasmablasts expand quickly after infection, with the population peaking within a week after the initial exposure (13) and contracting rapidly after viral clearance. It is this source of antibody that is therefore important for active clearance of virus in primary infection. In addition to plasmablasts, B cells within these extrafollicular responses can also differentiate into memory B cells (MBCs) (14). The distinct characteristics and role of these extrafollicular MBCs is currently an active area of investigation. A study using mice in which GC B cells had been conditionally knocked out found that MBCs were still detectable in the absence of germinal centers and were both low affinity and long-lived (15). Further investigation of the kinetics of B cell responses in a WT murine vaccination model confirmed the generation of both IgM and IgG expressing MBCs prior to the induction of germinal centers (16). Thus, extrafollicular responses are not only an important source of short-lived antibody-secreting cells but also long-lived MBCs that can contribute to future recall responses to infection.

In addition to their protective role in the context of acute infection, extrafollicular B cell responses have also been negatively implicated in settings of autoimmunity and chronic infection. A subset of cells actively secreting pathogenic autoantibody found in patients with systemic lupus erythematosus (SLE) have low mutational frequencies consistent with B cells that originate from extrafollicular responses (17). Another B cell subset implicated in autoimmunity are double negative (DN) B cells. Identified based on their lack of both IgD and CD27, DN B cells have been identified in circulation (18) and have been associated with autoimmune diseases such as SLE (19, 20). Given their low levels of somatic hypermutation, this population is

hypothesized to also derive from an extrafollicular response (18), and studies of patients with SLE found a clonal relationship between a subset of these cells (DN2s) and the pathogenic autoantibody secreting cells, providing further evidence for the contribution of these extrafollicular subsets to autoimmune phenotypes (20). These potentially pathogenic B cell subsets have also been identified in the context of chronic viral infection (21) and whether their presence in acute viral infections is a cause for concern is still an active topic of debate. Nevertheless, it is important that certain extrafollicular responses, especially these atypical B cell subsets, could be an indication of an aberrant immune response.

Germinal Center Responses

Within days after the initial induction of extrafollicular responses, structures known as germinal centers are formed within lymph nodes to facilitate the maturation and differentiation of additional effector B cell subsets, which are integral in the establishment of a protective humoral response (13, 22). Germinal centers have a unique organization consisting of two major compartments known as the light zone and the dark zone. The light zone acts as the primary center for B cells to test the affinity of their antigen receptor. This compartment contains various accessory cell types such as follicular dendritic cells and macrophages that act as antigen sinks in order to facilitate the repeated testing of antigen receptor affinity (22). The exact mechanisms for the positive selection of B cells in the light zone to differentiate and exit the germinal center is not completely understood but clearly involves a combination of the strength of both BCR signaling and engagement with T_{FH} cells present in the germinal center (2, 22). In contrast, the dark zone contains mainly B cells undergoing cell division to allow for both somatic hypermutation (the introduction of point mutations into the BCR) and class-switch (the swapping of the constant domain of the BCR) (22). These processes allow for the potential for the BCR to

increase its affinity for antigen, which is then tested in the light zone (22). Germinal centers continue to persist even after infection subsides, and their longevity appears strongly dependent on continued antigen availability (23, 24).

Memory B cells (MBCs) are the first effector B cells to differentiate and exit the germinal center (16). MBCs can be in constant circulation, similar to naïve B cells, but can also establish tissue residency in the lungs (25) and the gut (26). Upon reencounter with antigen, MBCs respond more quickly than their naïve counterparts and can be recruited to extrafollicular responses for rapid differentiation into antibody-secreting cells (27-29). Antigen re-encounter can also recruit MBCs back to germinal centers for further affinity maturation (30) with a preferential recruitment of IgM-specific MBCs observed in some studies (31). In contrast to MBCs, long-lived plasma cells (LLPCs) are produced in the late phase of germinal center reactions (16). LLPCs are terminally differentiated effector cells whose sole purpose is to constitutively produce antibody after antigen exposure. After exiting germinal centers, these cells primarily traffic to the bone marrow but can also be found in other tissue sites, where they act as long-lived sources of circulating antibodies (32, 33). The exact mechanisms of this long-term survival are still being investigated, but direct contact with bone marrow stromal cells as well as secreted cytokines (IL-6, APRIL) have been demonstrated to be integral in the development and maintenance of this population (34-36). The exact mechanisms that control the size and organization of this niche are also unclear, but recent studies using intravital imaging in murine models have begun to provide initial insights into the potentially dynamic nature of this compartment (37).

Antibody Effector Functions

While B cells can differentiate into many different subsets, the primary effector function remains the same: the production of antibodies, the secretory forms of the BCR. Dimeric in nature, antibodies consist of a heavy and light chain, and it is the combination of the variable regions of these chains that generates the diversity within the antigen binding domain (Fab) of the antibody (38). Additionally, the heavy chains form a constant or "crystallizable" domain (Fc) that mediates interactions with Fc receptors to promote various antibody-mediated effector functions (38). Different Fc regions, known as isotypes, are associated with various antibody responses, and the most commonly utilized in the response to viral pathogens are IgM, IgG, and IgA. IgM antibodies are commonly produced in the first exposure to an antigen; these antibodies tend to be low affinity but can demonstrate increased avidity due to the ability of the Fc region to form a pentameric structure with other IgM antibodies through the use of an additional J chain (38). As an infection progresses and clears, B cells participating in the response can class-switch from IgM to IgG and IgA. IgG antibodies can be further divided into 4 subclasses based on differential hinge regions which allow for more or less flexibility in their binding conformations as well as differential binding to FcγRs on innate effector cells (38). IgG antibodies are the most abundant with longer half-lives than other isotypes given their ability to interact with the neonatal Fc receptor (FcRn) that facilitates antibody recycling. IgA antibodies also exist in two subclasses and, similar to IgM, can exist in both monomeric and multimeric confirmations (38). Often associated with mucosal sites in the body, IgA antibodies are thought to be important in the initial defense against invading pathogens due to their presence at these barrier sites.

The classical effector function of antibodies is their ability to bind and inhibit pathogen entry into host cells, a process known as neutralization. Well-established in the context of

numerous viral infections, antibody neutralization is a main protective element of the adaptive immune response. However, recognition of additional antibody effector functions as protective components of the immune response is growing, especially in the context of diseases where antibody neutralization does not provide an accurate correlate of protection (38). These non-neutralizing functions are mediated through antigen-antibody complexes binding to host receptors that recognize the Fc domain (38, 39). A myriad of receptors recognize Fc regions and are expressed on a wide variety of innate cells including macrophages, neutrophils, and NK cells (39). Binding of antibodies to these receptors can stimulate innate effector functions such as the release of cytotoxic granules (40) or the phagocytosis of antigen-antibody immune complexes (41). Antibodies can also participate in the activation of the complement pathway (42). While there still remains gaps in our knowledge of the importance and role of antibodies in various infection settings, it is clear that antibodies play a critical role in protection from a wide array of pathogens.

SARS-CoV-2

In mid-December of 2019, cases of a severe pneumonia-like illness of unknown origin were reported in the city of Wuhan, located in Hubei Province, China (43, 44). Within the next month, the causative agent, a novel β-coronavirus that would be classified as SARS-CoV-2, was identified (43, 44). By February of 2020, SARS-CoV-2 had spread across the world with severe outbreaks occurring on multiple continents (45). Three months after the first reports from Wuhan, the World Health Organization declared SARS-CoV-2 a global pandemic, and an unprecedented shut-down of global trade and travel began (46). Two years after its initial identification, SARS-CoV-2 has infected over 430 million, caused over 5.9 million deaths, and continues to infect thousands worldwide every day (47). In addition to the direct health effects of the virus and its disease, termed COVID-19, both the devastating mental health (48, 49) and socioeconomic consequences of this pandemic have been unprecedented and will only be fully understood in the years to come. Despite these stark realities, past scientific research on endemic and pathogenic coronaviruses provided a solid foundation for scientists to understand this novel coronavirus threat, and current work by the global scientific community has exponentially increased both our understanding of the virus and its interaction with the immune system. These advances have paved the way for the rapid development of therapeutic and preventative strategies to combat SARS-CoV-2. However, many questions remain unanswered as we begin our third year of the pandemic.

SARS-CoV-2: Relationship to other human β-coronaviruses

SARS-CoV-2 is classified as a β-coronavirus and appears to share homology with viruses previously isolate from both horseshoe bats (50, 51) and pangolins (52, 53). The β-coronavirus family includes both animal and human coronaviruses with the primary reservoir of these viruses

existing in bats (54). Only four of the viruses in this family have been previously reported to infect humans: OC43, HKU1, MERS-CoV, and SARS-CoV-1 (43). OC43 was the first human coronavirus characterized along with its α-coronavirus counterpart, 229E (55), in the 1960s (56, 57). OC43 and a more recently characterized family member, HKU1 (58), are causative agents of often mild seasonal respiratory illnesses with children and older adults most susceptible to symptomatic infection (59-61). Both of these viruses circulate endemically in the global population with seasonally related peaks in infection burden (59-61). In addition to these endemic viruses, two other β-coronaviruses have been identified in the past two decades that are highly pathogenic. MERS-CoV, which causes Middle Eastern respiratory syndrome, was first detected in Saudi Arabia in 2012 (62). Unlike OC43 and HKU1, MERS-CoV can cause severe disease with over 800 deaths reported out of over 2,000 confirmed infections (63). Despite this high case fatality rate, human-to-human transmission of the virus has been limited (64) with the main reservoir of MERS-CoV thought to exist in camels (65). This unique viral reservoir aids in explaining the geographically isolation of the majority of cases of MERS-CoV to the Middle East (63), with the exception of one traveler-related outbreak in South Korea (66).

Out of the four coronaviruses known to infect humans, SARS-CoV-1 shares the greatest genetic homology with SARS-CoV-2 (79.6%) as well as significant similarities in viral pathogenesis, transmission, and clinical manifestation (67). Initial reports of pneumonia of unidentified origin began in late 2002 in Guangdong Province, China, with cases emerging in several countries by early 2003 (68, 69). Unlike SARS-CoV-2, public health measures combined with innate characteristics of the virus meant that the spread of the virus could be contained, and the epidemic ended in 2003 with 744 out of 8,098 persons infected succumbing to severe acute respiratory syndrome (SARS) (70). While there is evidence that the initial animal to human

transmission of SARS-CoV-1 occurred between humans and palm civets (71), subsequent sequencing suggests that the original reservoir of the virus was likely to be horseshoe bats (72). Human transmission of SARS-CoV-1 was found to be primarily through aerosolized droplets (73), although airborne transmission was suspected in one outbreak (74). Studies found that masking could effectively limit the spread of the virus in hospitalized settings (75). In regards to viral pathogenesis, SARS-CoV-1 entry into host cells was mediated through interactions of the receptor binding domain (RBD) on the spike protein (76), and the host cell receptor angiotensin-converting enzyme 2 (ACE2) (77). Preliminary work on preventative strategies to combat SARS-CoV-1 focused on the generation of humoral immunity towards the RBD on the spike protein as this site was found to elicit potent neutralizing antibodies (78, 79). The research conducted on SARS-CoV-1 and other human coronaviruses laid the groundwork for our understanding of both the viral characteristics of SARS-CoV-2 and the clinical pathology of COVID-19.

Viral Structure and Replication

The genome of SARS-CoV-2 is large as seen in other coronaviruses and encodes for wide variety of proteins including four structural proteins, 16 nonstructural proteins important for transcription and viral replication, and several additional accessory proteins (80, 81). The four structural proteins include the spike (S), nucleocapsid (N), membrane (M), and envelope (E) proteins (80, 81). Out of these four, the S protein appears to be the most critical for both viral entry to host cells and as a major target of neutralizing antibodies, and, as such, characterizing the structure of the S protein has been a primary focus of both virologists and immunologists in this ongoing pandemic. The S protein consists of two major subunits, the S1 and S2, the latter sharing 99% sequence identity with SARS-CoV-1 (80). The S1 subunit can be further broken

down into several domains, including the N-terminal domain (NTD) and the receptor binding domain (RBD), similar to SARS-CoV-1 (80-82). The S2 domain contains the fusion peptide, which becomes exposed upon proteolytic cleavage after receptor binding (82). Unique to SARS-CoV-2, the S1 and S2 subunits are separated by an additional proteolytic cleavage site, specifically a polybasic or furin cleavage site (FCS) (80, 82-84). This cleavage site allows SARS-CoV-2 to utilize ubiquitous host proteases to cleave the S protein, and the conformational change induced by this cleavage appears to enhance receptor binding and cellular entry (84, 85). A study comparing SARS-CoV-2 strains with and without the FCS demonstrated that the removal of the novel FCS reduces *in vitro* infectivity and attenuates disease severity in a hamster model (86). While further studies are needed to confirm whether the presence of this motif is a major driver of the pandemic nature of SARS-CoV-2, it is interesting to note that a polybasic cleavage site is considered one of the key determinants for highly pathogenic influenza strains (87).

Similar to other viruses that cause respiratory disease, viral replication of SARS-CoV-2 has been detected primarily in the upper respiratory tract early in the course of infection, with peak viral titers occurring within the first few days of symptomatic disease (88). Human-to-human transmission appears to be primarily facilitated through the spread of this localized virus through aerosolized droplets between individuals (89, 90). This transmission route has been confirmed in animal models, with direct and indirect contact leading to infection in ferrets (91,92), and through human observational studies (93, 94). However, the virus can be detected in other viscera (88) and can persist on surfaces for significant lengths of time (90). The contribution of these alternative methods of transmission is as yet unclear.

As stated previously, the S protein of SARS-CoV-2 is integral in mediating both viral binding and entry into host cells. The primary host receptor for SARS-CoV-2 is the same receptor that mediates SARS-CoV-1 viral entry, angiotensin converting enzyme 2 (ACE2) (43, 82, 85, 95-97). Recent studies have also suggested that viral binding and entry can be enhanced in the presence of NPR1, which is highly expressed on both epithelial cells and specialized CNS cell populations (98-100). Unlike SARS-CoV-1, binding of SARS-CoV-2 to ACE2 is enhanced through furin-mediated cleavage of the unique polybasic site which allows a conformational "opening" of the RBDs on the S trimer (84, 85, 101). Further conformational changes are mediated by host serine protease TMPRSS2 that allow for cleavage of the S2 subunit, which exposes the fusion peptide to mediate viral and host membrane fusion (85, 97). Co-expression of both ACE2 and TMPRSS2 on cell populations in the upper respiratory tract likely allow for their susceptibility to the virus, leading to the common respiratory symptoms seen in infection (102, 103). After entry into the host cell, the replication and release of SARS-CoV-2 progeny mimics that of other human coronaviruses. Briefly, the virus' positive single stranded RNA genome can be processed by host translational machinery to produce both structural and non-structural viral proteins (104). The translation of a large open reading frame, ORF1a/b, results in the production of both an RNA-dependent RNA-polymerase (Nsp12) and required accessory proteins, which allow for the amplification of the viral genome (81, 104, 105). Non-structural proteins (discussed below) can aid in the suppression of the innate immune response to allow for further viral amplification and infection of neighboring cells. Once genome replication and viral protein synthesis are complete, the new virions can then bud from the infected cell to further repeat the infection cycle.

Several characteristics of SARS-CoV-2 allow the virus to harness or evade host immunity to allow for increased viral fitness. Initial studies indicate that the expression of ACE2 is increased in the presence of interferon, which is highly expressed in the context of viral infections (103). Structural studies of the pre-fusion spike have additionally revealed that, in contrast to SARS-CoV-1, the receptor binding domains in SARS-CoV-2 exist in a closed conformational state, which could potentially contribute to the viral evasion of neutralizing antibodies (82, 84, 85, 97). Non-structural viral proteins also facilitate viral immune evasion. Characterization of Nsp1 determined that the protein binds and inhibits ribosomes, effectively halting translation of pro-inflammatory cytokines (106). Nsp10/16 have been found to participate in 5' capping of viral RNA, which allows the virus to evade detection by innate pattern recognition receptors (107). Together, these findings provide initial evidence of the ability of SARS-CoV-2 to effectively evade host immunity using strategies both unique to itself and shared between other coronaviral family members.

Disease Pathology

With over 430 million confirmed infections worldwide (47), clinicians have identified a common cluster of symptoms that occur in SARS-CoV-2 infection in the most individuals. However, a high level of symptom heterogeneity does exist within COVID-19 especially in individuals experiencing severe disease. In most mild to moderate cases of COVID-19, individuals experience symptoms an average of 5 days after initial viral exposure (108). Symptoms commonly include fever, malaise, dry cough, and sore throat, which generally resolve within 1-2 weeks of initial infection (109-112). However, a subset of patients can experience severe immunopathology, likely caused by "cytokine storm," that can lead to fatal acute respiratory or multi-organ failure (110, 111, 113, 114). Risk factors associated with more severe

COVID-19 outcomes are still being determined, but individuals with severe disease tend to include older adults, especially men, and those who have pre-existing conditions such as cardiovascular disease, diabetes, and other autoimmune conditions (110, 112, 115-118). Recent exome profiling of patients with mild and severe COVID-19 has also revealed a relationship between loss-of-function mutations in interferon- and toll-like receptor genes and more severe COVID-19 (119). Another survey of over 3,500 individuals with severe COVID-19 found that ~13% of severe COVID-19 patients had intermediate to high concentrations of autoantibodies against interferon, potentially dampening their initial immune response to infection with SARS-CoV-2 (120). Clearly, further work needs to be undertaken to determine if these genetic predispositions could be used to predict and manage susceptibility to severe COVID-19. In addition to both mild and severely symptomatic COVID-19, a portion of infected individuals appear to be completely asymptomatic. The rate of asymptomatic cases in the population has been difficult to determine given the overwhelming spread of the virus and a lack of robust testing infrastructure in many countries. From studies that were able to account for asymptomatic cases, it is known that, despite the lack of symptoms, these individuals can transmit virus early in infection (121), although their viral titers wane more quickly than in symptomatic COVID-19 (122).

Given that SARS-CoV-2 can be cleared in most individuals within 1-2 weeks of initial exposure (108), COVID-19 is generally thought to be an acute disease phenotype. Despite this, a myriad of long-term symptoms have been associated with SARS-CoV-2 infection, collectively termed “long COVID” (123). Not to be confused with individuals with persistent viral replication (124), individuals with “long COVID” tend to experience neurological symptoms pertaining to both cognitive and motor functionality accompanied by significant sensory loss

(smell and taste) and chronic muscular fatigue and exhaustion (123, 125, 126). Similar to individuals with severe COVID-19, risks factors for the development of these chronic symptoms are unclear, but the syndrome appears to disproportionately affect older females (126). Indeed, disease severity does not appear to be a consistent risk factor, with individuals recovered from both mild and severe COVID-19 going on to experience “long COVID” symptoms (127). Whether this disease manifestation is unique to SARS-CoV-2 is also unknown, as similar symptoms have been reported in the wake of other viral infections (128). Taken together, this initial evidence illustrates the extremely heterogeneity of COVID-19 disease and strongly supports the need for both therapeutic and preventative measures to alleviate the disease burden within the population.

HUMORAL RESPONSES TO SARS-CoV-2

Over the past two years of the pandemic, the global scientific community has made extraordinary strides in understanding the immune response to SARS-CoV-2. Much like our understanding of the virus itself, studies of other human coronaviruses provided a foundation for our understanding of the immune response to SARS-CoV-2 and the importance of neutralizing antibodies as a correlate of protection. As case rates rose across the world, study cohorts were established to both explore humoral responses in the acute phase of infection as well as the maturation and durability of these responses in the convalescent phase. Our understanding of this virus and its relationship with the immune system is continually evolving as the pandemic continues into its third year, but these initial studies have provided significant insight into the humoral response.

Lessons from Human Coronaviruses

Prior to the emergence of SARS-CoV-2, studies of both endemic and pathogenic human coronaviruses demonstrated coronavirus infection could generate effective neutralizing antibodies but that these antibodies appeared unlikely to contribute to long-term protective immunity. Two pivotal studies in the field were conducted in the late 1980s using a human challenge model of the α-coronavirus 229E (129, 130). The first study challenged and re-challenged volunteers with various lab-isolated strains of 299E in the course of a year, finding that only heterologous re-challenge resulted in re-infection as determined by both clinical symptoms and viral and antibody titers (130). A subsequent study using a similar homologous re-infection model found that homologous re-challenge resulted in re-infection in a subset of participants (129). While these results contradict the findings of the first study, this study additionally reported that, while the initial challenge promoted a rapid increase in neutralizing

IgG and IgA, these antibody titers waned to baseline levels by one year, supporting their detection of active re-infection in participants (129). However, none of the re-infected individuals became symptomatic, and the period of viral shedding was significantly reduced, which could aid in explaining the conflicting results between studies (129). While both of these studies were conducted on a small sample of individuals with pre-existing immunity to circulating endemic coronaviruses, these results provide a unique and important insight into the potential issues of the durability of humoral immunity to coronaviruses (129).

Additional epidemiological studies have further confirmed a pattern of antibody waning and continuous re-infection with endemic coronaviruses. Studies of respiratory infections in children have provided evidence that initial infections with endemic coronaviruses occur within the first few years of life (61, 131, 132) and that continuous reinfections occur throughout one's life (133) with higher antibody titers present in older adults (134). Coronaviral infections in adults are also not always correlated with increases in neutralizing antibody titers, suggesting that reinfections may boost non-neutralizing or less protective antibody populations (134). In contrast to reports of short-lived antibody responses, recent work to characterize antibody responses to endemic coronaviruses over ~7 months found stable antibody binding titers (135). Indeed, while heterologous reinfections are detected (132), epidemiological evidence in children suggests preferential infection with select coronaviruses (OC43, NL63) and raises the possibility that this infection pattern may provide heterologous cross-protection (136). Although further work is needed to confirm this theory, these studies demonstrate the complexity of the humoral response to these endemic human coronaviruses.

In the wake of the emergence of both SARS-CoV-1 and MERS-CoV, many studies sought to determine whether the durability of the humoral response to these pathogenic human

coronaviruses resembled that of the response to endemic coronaviruses. Studies following individuals recovered from SARS-CoV-1 found that antibody titers peaked 2-4 months after exposure and significantly decreased in the following months; however, neutralizing antibodies titers were still detectable in donors up to 3 years after infection (137-140). Studies assessing the durability of neutralizing antibodies against MERS-CoV show similar results, with neutralizing antibodies persisting in MERS recovered patients almost 3 years after infection (141). Limited studies in MERS recovered patients have also demonstrated a correlation between disease severity and antibody titers with individuals with mild or asymptomatic cases becoming seronegative 3-12 months after infection (142, 143).

Given the extensive pre-existing immunity to endemic coronaviruses, a major question remains as to the role of this immunity in pathogenic coronaviral infection. A study comparing individuals infected with either SARS-CoV-1 or an endemic coronavirus found that, while a subset of individuals had increased antibody towards endemic coronaviruses after SARS-CoV-1 infection, no SARS-CoV-1 cross-reactive responses were observed in individuals after infection with an endemic coronavirus (144). Furthermore, a study examining cross-reactivity between SARS-CoV-1 and animal coronaviruses demonstrated that the antibodies that cross-reacted between viruses were N- rather than S- binding, which calls into question the protective nature of these boosted antibodies (145). While further work is needed to explore these cross-boosted antibody responses, the initial evidence indicates that these responses are likely not a source of protection against pathogenic coronaviral infection.

In addition to insight into the dynamics of the humoral response, animal studies evaluating SARS-CoV-1 and MERS-CoV vaccine candidates have provided strong evidence of the immunodominance of the S protein to elicit potent neutralizing and protective antibody

responses. Immunization of a hamster model with vectors containing the four major structural protein of SARS-CoV-1 (S, E, M, N) found that only constructs containing the S protein elicited neutralizing antibodies and provided protection against viral challenge (78). Additional studies of both inactivated and vector-based vaccines in mice supported this finding with the induction of neutralizing S-specific antibodies providing protection against viral challenge (146, 147). Further characterization of these S-specific antibodies demonstrated that antibodies targeting the receptor binding domain (RBD) were the major drivers of neutralization in both a rabbit immunization model and in plasma isolated from SARS-CoV-1 infected humans (148). Potent monoclonal antibodies were found to bind to the RBD site, although antibodies binding outside this site were also found to be neutralizing (148, 149). More recent studies of MERS-CoV have also found that vaccination with an mRNA construct encoding the S protein elicits neutralizing antibodies and provides protection in a transgenic mouse model (150). In addition to the antibodies targeting the RBD, a potent NTD-targeting antibody has also been identified in the context of MERS-CoV that acts to block the conformational shift between pre- and post-fusion and appears have additive protective effects when combined with RBD-specific antibodies (151). These initial studies of the antigen specificity of the antibody response to SARS-CoV-1 and MERS-CoV provided the foundation for our understanding of immunodominant antibody targets in the context of SARS-CoV-2.

Antibody Responses to SARS-CoV-2 Infection

The kinetics and durability of the antibody response induced by SARS-CoV-2 infection were rapidly characterized in the months following the start of the pandemic. Seroconversion within acutely infected individuals occurred more rapidly than in SARS-CoV-1 patients (139) with the majority of infected individuals seroconverting within the second week post symptom

onset against the primary immunogenic coronaviral proteins (S and N) (152-154) with seroconversion correlating with decreases in viral RNAemia (154). Interestingly, infected individuals largely seroconverted across all isotypes (IgM/IgG/IgA) at approximately the same time (152) in contrast to the traditionally observed stepwise seroconversion to IgM early in infection, followed by seroconversion to class-switched IgG and IgA later in infection. Greater disease severity has been consistently correlated with higher antibody titers (152, 154-156), with increased age also being correlated in certain cohorts (156). Additionally, a report comparing outpatient to inpatient COVID-19 observed higher levels of N-specific antibodies as compared to RBD-/S-specific in individuals with more severe disease, suggesting that a bias towards non-neutralizing antibodies could be driving disease in certain individuals (154). While initial reports suggested that individuals with mild (157) or asymptomatic (154, 158) disease were more likely to become seronegative in the months following infection, larger cohort studies have reported that even mild infections are able to induce binding and neutralizing antibody titers still detectable between 3 and 8 months after infection (135, 155, 159-162). Modeling of this antibody decay post-infection has been contradictory with some groups reporting a significant constant decrease in binding and neutralizing IgG titers (153, 159, 160) while others have presented a more nuanced model supporting a biphasic decline in titers where titers initially fall rapidly and then stabilize into a slower rate of antibody decay (135). The majority of studies find a rapid waning of IgM titers (160, 162) with some studies reporting a more durable IgA response (160), although evidence for this is not consistent (162). Long term follow-ups of these infection cohorts are currently underway, although the widespread availability and administration of vaccines will likely limit the number of long-term studies tracking infection-induced antibody titers.

Similar to SARS-CoV-1, neutralization of SARS-CoV-2 has been positively correlated with S-specific antibody titers and even more strongly correlated with RBD-specific titers (135, 153, 156, 159-161, 163). Indeed, removal of RBD-binding antibodies from plasma has been reported to significantly reduce plasma neutralization in SARS-CoV-2 infected individuals (164, 165). In-depth characterization of antibodies cloned from S- and RBD-specific plasmablasts and MBCs further supports the strong relationship between RBD-reactivity and neutralization, with the majority of neutralizing antibodies identified being RBD-specific (156, 162, 164, 166-171). RBD-specific antibodies are often only specific to SARS-CoV-2 (153, 172), but some studies have identified RBD-specific antibodies that are cross-reactive between SARS-CoV-1 and -2 (164, 173) as well as between SARS-CoV-2 variants that have emerged over the course of the pandemic (156, 168, 169, 174). Studies of these RBD-binding antibodies have been found to target several distinct epitopes within the RBD and can directly or indirectly block ACE2 binding (168,156, 164, 170). A structural study of 8 antibodies derived from COVID-19 convalescent individuals delineated four unique categories of RBD-binding (170). Antibodies directly blocking the ACE2 binding site were found to be able to bind either only RBD in the "up" position (Class I) or RBD in both the "up" and "down" position (Class II) with antibodies binding outside the ACE2 binding site exhibiting similar conformational dependencies (Class III & IV) (170). Further structural analyses of the ACE2 binding site, the receptor binding motif (RBM), has revealed that two unique epitopes exist within this site, delineated by their ability to be recognized in either the "open" or "closed" spike confirmation (165). Many potently neutralizing RBD-specific antibodies have been found to interact directly with the ACE2 binding site (169, 172, 175), but neutralizers have also been found to engage with other epitopes within the RBD (156, 168, 174), with some targeting quaternary structures (175). The potential of RBD-

specific antibodies as both prophylactics and therapeutics was quickly demonstrated in animal models (164, 169, 171), and several are currently in use as mono- or combinational antibody therapies for COVID-19 (176-178). Taken together, RBD-specific antibodies are clearly a critical component of the neutralizing antibody response to SARS-CoV-2 infection and have the potential to be actively utilized in preventative and therapeutic efforts to combat the pandemic.

Additional domains within S protein have also been found to elicit neutralizing antibodies, including the N-terminal domain (NTD) and the S2 subunit. While less immunodominant than RBD, NTD-specific antibodies can potently neutralize SARS-CoV-2 both *in vitro* and *in vivo* (164, 166, 167, 174, 179, 180). However, unlike RBD-specific antibodies, neutralizing NTD-specific antibodies appear to only target a single "super-site" within the NTD, likely restricted by the significant glycosylation of the NTD region (180). Unsurprisingly, preliminary studies indicate that NTD-specific antibodies are highly susceptible to mutational variation and are unlikely to be able to neutralize across variants or distinct family members (166). Thus, while NTD-specific antibodies can contribute to the antibody repertoire elicited by SARS-CoV-2 infection, these antibodies are unlikely to contribute to a dominant neutralizing response. In contrast to both the NTD and RBD, few neutralizing antibodies targeting the S2 domain have been described, and those that have been found often demonstrate weak neutralizing potency (169). Despite this, significant levels of S2-specific antibodies and MBCs have been detected in SARS-CoV-2 recovered individuals (164, 181, 182). Given the high level of conservation of the S2 subunit between coronaviruses, it is likely that these S2-specific antibodies are being derived from B cell pools established in prior endemic coronaviral infections (80). While it is unlikely that these antibodies contribute significantly to the

neutralizing antibody response, the potential protective role of S2 antibodies through other non-neutralizing effector functions remain unclear and warrants further investigation.

Another area that requires further exploration is whether SARS-CoV-2 cross-reactive antibodies exist within the antibody repertoire established by previous coronavirus infections and, if they do, what their role is in SARS-CoV-2 immunity. Initial analyses attempting to detect SARS-CoV-2 antibodies in pre-pandemic individuals have yielded contradictory results. While some groups have mainly detected non-neutralizing antibodies reactive to SARS-CoV-2 S and N proteins (181, 183), another group found significant SARS-CoV-2 neutralizing titers in uninfected individuals (184). A consensus between these groups is that the S-reactive subset of these antibodies appear to be targeting the highly conserved S2 domain (184). Individuals expressing these cross-reactive antibodies tended to have higher titers of antibodies towards endemic β-coronaviruses than individuals in which this population was absent (183). S- and S2-specific antibodies against OC43, HKU1, and SARS-CoV-1 have been found to be elevated after exposure to SARS-CoV-2, suggesting a role for SARS-CoV-2 in activating pre-existing memory B cell populations (135, 154, 181-183). Further supporting this hypothesis, a study of 377 antibodies derived from COVID-19 convalescents found that the small percentage of antibodies that cross-reacted with endemic coronaviral S proteins tended to have higher levels of somatic hypermutation than their SARS-CoV-2 specific counterparts (164). Another study tracking recovered individuals over time found cross-reactive MBC that bound endemic S protein in the acute phase of infection; however, this population disappeared at later timepoints, and the long-term impact of this cross-reactive population remains unclear (161). While these initial studies do not strongly suggest that pre-existing immunity provides significant cross-protection to SARS-CoV-2, a comparative study of individuals with and without a confirmed endemic

coronaviral infection in the 5 years prior to the pandemic found that individuals more recently infected had a lower risk of severe COVID-19 (185). Additionally, the presence of non-neutralizing cross-reactive antibodies raises potential concerns about antibody-dependent enhancement (ADE), a phenomenon in which non-neutralizing antibodies can potentially aid in the trafficking of the infecting virus to immune cells to cause enhanced disease pathology (186). Initial studies of ADE in SARS-CoV-2 have demonstrated that, while certain RBD- and NTD-targeting antibodies have ADE activity *in vitro* (168), the phenomenon is not seen in either mouse or NHP models of SARS-CoV-2 (167). While a clear relationship exists between pre-existing coronaviral immunity and SARS-CoV-2 infection, the overall role of this response in influencing the protective response to SARS-CoV-2 is still being determined.

B Cell Responses to SARS-CoV-2 Infection

Infection with SARS-CoV-2 is accompanied by both rapid expansions of antibody-secreting cells (ASC) and the induction and establishment of an antigen-specific memory B cell pool. These adaptative B cell responses are clearly important in COVID-19, as individuals lacking activated B and T cell responses have been found to have poor disease outcomes (187). Robust plasmablast expansions are often observed in the context of viral infection (188), and significant plasmablast expansions have been observed in multiple cohorts of individuals with acute SARS-CoV-2 infection (187, 189-193). Highly elevated plasmablast frequencies have been correlated with severe disease (190, 192), and a high-dimensional flow analysis of over 100 COVID-19 patients associated increased plasmablast frequencies with an immunotype linked to severe disease that included highly activated CD8 T cells and low frequencies of circulating T_{FH} cells (187). The same study additionally found that plasmablast frequencies were only weakly correlated with circulating T_{FH} cells and found no correlation with antibody titers in these

patients (187). Additional characterization of the plasmablast response in severe individuals revealed that this population was characterized by high levels of somatic hypermutation coupled with large clonal expansions (192). However, other studies have also reported increases in germline or unmutated plasmablasts in individuals with severe disease (190), which suggests significant patient to patient heterogeneity. While the plasmablast response in viral infections tends also to be highly antigen-specific (188), a characterization of 219 plasmablast-derived monoclonal antibodies identified only 32 S-specific antibodies, of which only 14 had detectable neutralizing activity (191). This initial evidence suggests that the plasmablasts induced in SARS-CoV-2 infection may originate from multiple pools of circulating B cells and potentially have a more complex role in COVID-19 than in other viral infections.

In contrast to their plasmablast counterparts, strong evidence exists supporting the protective role of MBCs in SARS-CoV-2 infection. Mild COVID-19 disease is associated with increased total MBCs as compared to individuals with severe disease (187, 190), and more rapid recoveries are observed in individuals with higher numbers of both IgM^+ and switched MBCs (194). Additionally, total MBC responses positively correlate with circulating RBD-specific antibody titers, suggesting a relationship between the two populations (194). Methods to detect SARS-CoV-2 S- and RBD-specific MBCs were quickly established, and SARS-CoV-2-specific MBCs can be detected early in infection (155). Interestingly, both S- and RBD-specific MBCs increase over the months after infection with IgG being the dominant isotype utilized (135, 155, 160-162). While the majority of these antigen-specific MBCs display a resting phenotype, a significant portion continue to display markers associated with activation (CD71), suggestive of a sustained immune reaction (161). While antigen-specific IgM MBCs are detectable early in the response, these cells appear to be short-lived in contrast to their IgG counterparts (135, 155).

Similar to antibody titers, higher levels of antigen-specific MBCs were positive correlated with disease severity (155).

The evolution of MBC response to SARS-CoV-2 has been an area of great interest to determine if the increases in the antigen-specific MBC population over time are linked to the generation of antibody repertoires of greater breadth and potency. Many of the initial studies of antibodies generated early in infection revealed extremely low levels of somatic hypermutation, indicating a primarily *de novo* response (169, 172, 174, 175). A comparative study of antibodies derived from RBD-specific MBCs at ~1 and ~6 months after infection offered initial insight into the further development of this response (160). The group found that, while the mutational load increased over time, clonal expansion decreased over time, and several clonal groups evident early in convalescence had disappeared at later timepoints (160). Additionally, antibodies derived from later in convalescence were more likely to bind and neutralize SARS-CoV-2 variants (160). Similar trends of increasing mutational frequency (195) and antibody potency (161) have been observed in other cohorts. Comparisons of clonality between early infection ASCs and convalescent stage MBCs have found little overlap between these populations, suggesting that the initial extrafollicular response is distinct from the later germinal center reactions that generate the durable antigen-specific MBC population (161). To further support this theory, distinct mutational signatures for S- and RBD-specific MBC populations have been identified with the S-specific population containing MBCs with both high and low mutational signatures, suggesting that this population originated from two unique pools of B cells (161). Taken together, these studies provide initial evidence for sustained germinal center activity, and thus humoral immune maturation, long after viral clearance.

In addition to the expected plasmablast and memory B cell responses, a substantial number of studies have also found significant levels of "atypical" B cells in the context of severe COVID-19. A high-dimensional flow cytometry analysis of B cell responses to SARS-CoV-2 found a unique B cell signature associated with extrafollicular responses present in individuals with severe disease (196). These individuals had both expansions of antibody-secreting cells (ASCs) as previously noted, but also increased numbers of atypical B cells, more specifically double negative (DN) B cells which are characterized by their lack of typical B cell markers (CD10, CD27, IgD) and differential expression of CD11c and CD21 (196). Elevated levels of DN B cells have been now been identified in multiple COVID-19 cohorts with the highest frequencies found in individuals with poor disease outcomes (189, 192, 197). In addition to their presence in circulation, work characterizing the structure and cellular make-up of lymphatic organs (lymph nodes and spleens) in individuals who succumbed to COVID-19 found similar significant populations of both ASCs and DN B cells in these tissues (198). A lack of germinal center structures in addition to significant decreases in both germinal center associated B and T_{FH} ($Bcl6^{+}$) cells was also observed in these individuals, suggesting that an inability to mount germinal center reactions coupled with ineffective extrafollicular responses could have contributed to mortality (198). While the exact role of these DN B cells in the context of COVID-19 needs to be further explored, the well-documented presence of these B cell populations in both settings of autoimmunity (19, 20) and chronic infection (21) suggests that these cell populations may have a role in COVID-19 disease pathology. It is also interesting to note that there have be clinical reports of autoimmune disease developing after SARS-CoV-2 infection (199) – whether these atypical B cell responses are involved in this disease outcome remains to be elucidated.

COVID-19: TREATMENT AND PREVENTION

Current SARS-CoV-2 Treatments

No consistent guidelines have been established in the treatment of COVID-19, but several therapeutic approaches are currently under investigation and available under emergency usage globally. Therapeutic options can be broken down into three major categories including immunomodulatory therapies, anti-viral pharmaceuticals, and antibody-mediated therapies. In individuals who are hospitalized with severe COVID-19, steroids and immunosuppressants were initially used in an attempt to dampen the pathological "cytokine storm" associated with these symptoms. The efficacy of these treatments is variable, and their usage is currently only recommended for those who are already hospitalized and under intensive care (200,201, 202). At the onset of the pandemic, several anti-viral drugs were repurposed for COVID-19 clinical trials based on promising *in vitro* (203) and *in vivo* (204) data. Unfortunately, many of these anti-viral drugs had conflicting reports of clinical efficacy (205,206), with many no longer recommended for treatment of COVID-19 (206). At the beginning of this year, Pfizer released a two-drug antiviral regime, which reports robust efficacy in preventing severe COVID-19 if taken within the first several days of infection (207). Further data will have to be gathered to determine the efficacy of this treatment in wider practice, but the limited availability and high-cost of these drugs as a whole restrict their usefulness in combating the global pandemic.

Antibody-mediated therapies take advantage of the ability of the antibodies elicited through infection to neutralize SARS-CoV-2 and attenuate disease. These therapies include the use of convalescent plasma from patients recovered from COVID-19 (208) and several monoclonal antibody therapies where one or more antibodies derived from patients recovered from SARS-CoV-1 (177, 209) or SARS-CoV-2 (210) are administered to resolve disease. The

efficacy of these therapeutic strategies has been mixed with positive clinical outcomes mainly in high-risk patients early in disease progression (177, 200, 210, 211). However, like anti-virals, both plasma and monoclonal antibodies are limited in their supply, and monoclonal antibodies are only available in IV formats at high-cost to providers. Monoclonal antibody therapies, especially those reliant on a monotherapy approach, are also susceptible to viral escape mutants as evidenced by the severely reduced efficacy of the majority of these therapies against the newly emerged Omicron variant (212). Thus, preventing the continued spread of SARS-CoV-2 infection through clear public health guidance, easy access to testing, and safe and free vaccination is the best strategy to resolve the ongoing pandemic.

SARS-CoV-2 Vaccination Strategies

Vaccination against viral pathogens has been responsible for not only the complete eradication of smallpox (213) but also significant declines in morbidity and mortality for a host of childhood diseases (214). With the emergence and rapid spread of SARS-CoV-2 across the globe, the development of a safe and effective vaccine as a tool of prevention and attenuation of disease became a worldwide goal. Pre-clinical work to develop vaccines against SARS-CoV-1 and MERS-CoV sped efforts to develop a SARS-CoV-2 vaccine, allowing clinical trials using several vaccine platforms to begin within the first months of the pandemic. As of the current moment, there are over 100 vaccine candidates in various stages of clinical trials with 33 vaccines approved for use in select countries across the world.[1] Vaccine developers have utilized both traditional and next generation platforms in the rapid generation of these vaccines. Three vaccines (BBIBP-CorV, Coronavax, and Covaxin) use an inactivated virus platform: the SARS-CoV-2 virus is propagated in Vero cells and inactivated with the sterilizing compound, β-

[1] Latest information on SARS-CoV-2 vaccines can be found at https://covid19.trackvaccines.org/

propiolactone (215-217). Two vaccines produced by Novavax use a protein-subunit platform, where full length S protein derived from SARS-CoV-2 is stabilized in a nanoparticle that is injected with M1 adjuvant (218). While none are yet WHO/FDA approved, several live attenuated vaccines, another traditional vaccine platform, are currently in clinical trials. The safety and efficacy of all of these platforms is extremely well-established and utilized for commonly administered childhood and adult vaccines (219).

In addition to vaccines developed using traditional methods, the SARS-CoV-2 pandemic has accelerated the widespread testing and usage of vaccines developed with next-generation platforms. These platforms include the use of DNA or RNA immunogens as well as the use of adenoviral or pseudoviral vectors. The benefits of DNA and RNA-based vaccines have generated much interest in the scientific community prior to the emergence of SARS-CoV-2. Unlike their traditional counterparts, these vaccines are not dependent on specific viral or protein culture systems that can be difficult to both establish and scale for vaccine production; these sometimes complex systems can also limit the adaptability of vaccines when new viral variants arise. A prime example is the current influenza vaccine, manufactured using a traditional inactivated virus platform, which has a 6-9 month lead time before the vaccine is ready for distribution (220). The weakness of this system is readily evidenced by significant reductions in vaccine efficacy during seasons when a mismatch has occurred between the circulating and vaccine strains (221, 222).

Several next generation platforms were used in the development of vaccines against SARS-CoV-2 with many now approved for global usage. The two platforms that have generated widely available vaccines are the adenoviral vector and mRNA platforms, although several DNA-based vaccines are in various stages of clinical testing. The two WHO-EUL approved

adenoviral vaccines are produced by Oxford-AstraZeneca (COVISHIELD) and Janssen (J&J). These vaccines rely on either human (Ad26) or chimp (ChAdOX1) adenoviral vectors engineered to express modified S protein to elicit a protective immune response (223). Results from clinical trials demonstrated that these adenoviral vector-based vaccines could generate both neutralizing antibodies and T-cell based immunity with reported efficacies up to 64.3% in Phase III clinical trials (224-226). Despite the positive clinical results, the real world deployment of these vaccines has been variable due to issues with rare cases of severe blood clots (227) and reduced efficacy against emerging SARS-CoV-2 variants (228). The final two WHO-EUL vaccines are the most widely available vaccines within the United States; these vaccines are Moderna's mRNA-1273 and Pfizer-BioNTech's Comirnaty, both of which rely on an mRNA platform.

The Success of SARS-CoV-2 mRNA vaccines

Research on mRNA vaccines has been ongoing since the 1990's when initial studies provided the proof-of-principle that mRNA could be used to generate a protein of interest when injected directly into mice (229). While the potential of mRNA to be used throughout the medical field in both preventative and therapeutic settings was recognized, several scientific advances were necessary for the deployment of mRNA in a clinical setting. Foreign, unmodified mRNA is an inherently unstable, easily degraded, and can trigger innate immune response through TLR activation (230). The modification of mRNA sequences through polyadenylation (231) and sequence optimization, including increases in G-C content (232) and optimization of codons (233), enhanced stability of *ex vivo* mRNA products and the efficiency of protein production. The discovery that the modified nucleosides present in mammalian mRNA acted to suppress host innate immune activation (234) allowed researchers to incorporate similarly

modified nucleosides (i.e. pseudouridine) into engineered mRNA products to dampen uncontrolled innate immune activation and improve protein production (235,236). Advances in purification technologies to remove highly immunogenic double-stranded RNA from engineered mRNA preparations also decreased the immune-activating properties of this substrate (237). Implementation of lipid-nanoparticle technology in the delivery of mRNA products marked an important improvement to the translational efficacy of mRNA vaccines (238, 239). Taken together, these advancements provided the foundation for the rapid development of two safe and efficacious mRNA vaccines against SARS-CoV-2 that were available for use within a year of the initial emergence of the novel virus.

Ongoing studies to develop an mRNA vaccine encoding a pre-fusion stabilized S protein against another pathogenic coronavirus, MERS-CoV, provided the basis for mRNA-1273 (150). mRNA-1273 is delivered in two doses of 100 μg 28 days apart (240, 241). Phase III clinical trials determined mRNA-1273 was 93-94% effective at preventing COVID-19 disease, although this efficacy was lower for prevention of asymptomatic infections (240, 241). Comirnaty utilizes an almost identical mRNA immunogen as mRNA-1273, but is delivered at a lower dose (30 μg) with the two doses being 21 days apart (242). Initial studies demonstrated that one dose of vaccine yielded weakly neutralizing antibody responses (243) and that two doses were required for induction of robust neutralizing titers and T_H1 responses (244). Longitudinal cohort studies have demonstrated an initial vaccine efficacy of 88% within the first month but found that efficacy wanes to 47% 5 months after vaccination; interestingly, these efficacies were approximately the same when infections with SARS-CoV-2 variants were separated, suggesting the induction of a broadly protective response (245).

While the differences between the composition of the two mRNA vaccines are seemingly minor, recent reports have illustrated differences in the immunity generated by each vaccine. A recent report comparing the efficacy of the two vaccines in veterans demonstrated that, while both provided substantial protection from severe disease and hospitalization, mRNA-1273 conferred a slight advantage in protection over Comirnaty (246). This finding was echoed in a recent study of COVID-19 hospitalization, where individuals vaccinated with Comirnaty experienced a slight increase in breakthrough infection and hospitalization (247). Furthermore, a comparison of vaccine efficacy demonstrated that, while efficacy remains robust in mRNA-1273 vaccinated individuals, dropping from 93 to 92% 4 months after vaccination, individuals vaccinated with Comirnaty experienced a steeper decline with efficacy dropping from 91 to 77% after 4 months (248). Although a small healthcare worker study of S-specific antibodies revealed higher titers in mRNA-1273 vaccinated participants as compared to Comirnaty (249), in-depth comparisons of the cellular and serological responses of the two vaccines are needed to determine the potential causes of these observed efficacy differences. It is important to note that, from a public health perspective, these reported differences in efficacy are minor compared to the substantial difference in infection and hospitalization between unvaccinated and vaccinated individuals (247) but further exploration could give insight into the durability and breadth of these vaccines to protect against future SARS-CoV-2 variants.

THE EVOLUTION OF SARS-CoV-2

Many widely circulating human viruses are able to mutate to both increase viral fitness and escape pre-existing immunity within the population. An excellent example of this phenomenon is influenza, which uses an error-prone polymerase mechanism to induce mutations that allow it to evade neutralizing antibodies established during previous infections (250). In this way, influenza is able to ensure its continued endemic circulation within the population. Whether the SARS-CoV-2 pandemic will eventually transition to an endemic situation similar to influenza is still an open question, although its ability to persist in the face of an increasingly immune population suggests that this fate is likely. Previous studies of endemic coronaviruses provide preliminary evidence that coronaviruses can undergo genetic drift and positive selection, and the emergence of several variants of SARS-CoV-2 has made it clear that SARS-CoV-2 can utilize similar strategies for continued circulation.

Endemic Coronavirus Evolution: Evidence & Mechanisms

It has been established that genetically distinct strains of endemic coronaviruses have emerged and replaced original viral strains over years of circulation (251, 252). A comparative study of genetic drift in endemic coronavirus and influenza revealed similar, if slower, patterns of genetic drift in coronaviruses with the receptor binding domains being centers of mutational accumulation (253). The decreased rate of mutational acquisition could be due to the unique proofreading exonuclease (Nsp14) encoded in the coronaviral genome (104). Indeed, studies of coronaviruses with this exonuclease removed from the genome are more susceptible to mutagens *in vitro* (254). As in influenza, pre-existing immunity has also been shown to drive the mutational shift of endemic coronaviruses. Studies of mutational changes to the receptor binding domain of 299E over its 40+ years in circulation found evidence that the cumulative mutations

have allowed the virus to both escape antibody neutralization and increase receptor affinity (252). In support of this finding, a recent study of 299E reactive plasma found that, while individuals could neutralize the predicted infecting strain, antibodies were ineffective against strains in circulation ~10 years later (255). In addition to the receptor binding domain, the NTD has also been found to be a major site of positive selection and is extremely variable both within and between coronaviruses (256).

SARS-CoV-2: The Emergence of Viral Variants

Early *in vitro* studies of both monoclonal antibodies and convalescent plasma highlighted the mutability of key residues that could potentially lead to the immune escape of SARS-CoV-2 (257-259). Studies using yeast-display libraries of recombinant RBD containing single amino acid mutations demonstrated that, despite mutational restrictions dictated by protein confirmation and receptor binding, many key residues targeted by neutralizing antibodies could be mutated to facilitate immune escape (258). A concurrent study by the same group found that residue E484 within the RBD was a major determinant of plasma neutralization and that mutations to this site yielded severely reduced neutralization in plasma from recovered individuals (259). Additional sites within the RBD, including a ridge structure and the receptor binding motif (RBM) loop, were also identified as critical sites (259). Given the rapid and continued spread of SARS-CoV-2 throughout the world, it is unsurprising that several variants of SARS-CoV-2 containing these predicted mutations have arisen and outcompeted each other to become the dominant circulating strain.

The mutation of an aspartic acid to a glycine residue at position 614 was noted early in the pandemic (260). Viruses harboring this mutation quickly dominated the wild-type strain, leading to increased viral transmissibility (260). In the fall of 2020, two variants were

independently identified in the United Kingdom (261) and South Africa (262). The U.K. variant (B.1.1.7; Alpha) included a mutation in the S protein (N501Y) that was correlated with increased viral transmission and disease severity (263-266), but resulted in modest reductions in immunity established in infection or vaccination (267, 268). The S.A. variant (B.1.351; Beta) contained both the previously described N501Y and additional mutations at K417(N) and E484(K) (262). Studies of this variant confirmed that neutralization against this variant was reduced in plasma from infected and vaccinated individuals (262, 268-271), but the global spread of this variant was curtailed as it was outcompeted by the U.K. variant (272). A viral variant (P.1; Gamma) with a similar mutational footprint arose in Brazil in late 2020 (273), but, despite increased fitness (271, 273, 274), was not globally widespread (272). In March 2021, a new clade of variants arose in India, contributing to an unprecedented surge of cases in the country (275). A variant from this clade (B.1.617.2; Delta), containing an L452(R) mutation in the S protein (276), spread globally, displacing all pre-existing variants as the dominant SARS-CoV-2 strain (272). While neutralizing antibodies elicited from exposure to the original strain were weaker against this variant (276, 277), pre-existing immunity in the population aided in attenuating severe disease and preventing hospitalization (278). In addition to the four variants described, several more have been identified over the course of the pandemic (272), illustrating the adaptability of this viral threat. As this dissertation is being prepared, yet another variant of concern has emerged from South Africa (B.1.1.529; Omicron) and is rapidly becoming the dominant SARS-CoV-2 strain (272) – the impact of this variant and its implications for future vaccine and therapeutic strategies will be discussed in Chapter 4.

SUMMARY

B cells play an extremely important role in the context of viral infections. Actively recruited by viral-induced immune mediators, both rapid extrafollicular plasmablast responses and sustained germinal center reactions contribute to viral clearance and protection from re-infection. With the emergence of SARS-CoV-2 in 2019 and its related disease, COVID-19, understanding the role of B cells in the context of this novel threat remains critical. Previous work on both endemic and pathogenic human coronaviruses not only contributed significantly to our foundational understanding of SARS-CoV-2 as a virus but also the ways in which B cells could mediate a functional, protective, and durable immune response. Over the last two years, researchers across the globe have worked tirelessly to build upon this foundation and provide necessary insight into the specifics of both the cellular and antibody response to SARS-CoV-2 infection and vaccination.

Initial efforts to understand the humoral immune response to SARS-CoV-2 focused on fully characterizing this response in individuals with acute infection. In Chapter 2, we examine the cellular and serological responses to acute SARS-CoV-2 infection in patients with severe COVID-19. We found that plasmablasts were significantly expanded accompanied by a parallel reduction in the total MBC population; despite this reduction, activated RBD-specific MBCs were readily detected 1-2 weeks after symptom onset. Accompanying this detection of antigen-specific MBCs, RBD-, S-, and NP-specific IgG and IgA antibodies were detected in the majority of individuals during the same time period, with IgA titers appearing lower in individuals sampled further from symptom onset. Neutralizing antibody titers were robust and positively correlated with both RBD- and S-binding. In further support of this relationship, the main contributors to circulating neutralization were RBD-binding antibodies in the majority of

individuals; however, a subset of individuals retained 30% or more of their neutralizing activity in the absence of RBD-binding antibodies, suggesting a potentially important role for non-RBD-binding antibodies in certain individuals. This study contributed to our understanding of the humoral response to infection and echoed the findings of other groups examining these responses in unique cohorts across the world.

With the wide availability of SARS-CoV-2 mRNA vaccines in the U.S., efforts shifted to comparing vaccine-generated humoral responses to those generated in infection and how significant levels of pre-existing immunity to SARS-CoV-2 in the population alter the vaccine response. In Chapter 3, we present our findings from a longitudinal cohort of recovered and naïve individuals who received mRNA vaccination and were sample at seven timepoints up to six months after vaccination. We determined that pre-existing immunity to SARS-CoV-2 infection influences not only the kinetics and durability of the vaccine-induced humoral response but also significantly alters the breadth and potency of the antibody repertoire generated through vaccination. Additionally, we found that antigen-specific MBC responses in naïve vaccinated individuals closely paralleled the responses in individuals with primary SARS-CoV-2 infection and that vaccination in these individuals was able to generate potent antibody responses similar or better to those generated in infection. While the two mRNA vaccines performed similarly, we did note that naïve Moderna-vaccinated individuals retained a greater amount of neutralization upon removal of RBD-binding antibodies, suggesting that these two vaccines may generate antibody repertoires with differing epitope specificities. This study joins a growing number of reports characterizing the robust humoral responses in vaccinated individuals.

Taken together, our work over the last two years to characterize the humoral response to both infection and vaccination against the novel coronavirus SARS-CoV-2 has significantly

contributed to our understanding of the humoral response to viral infection and has added to a rapidly growing body of scientific literature aimed at mitigating the ongoing global pandemic. However, key questions remain. Chapter 4 will highlight some of these questions including the potential role of B cells in apparent viral-induced autoimmunity and the influence of both pre-existing immunity and vaccine type on the antibody repertoire. Additionally, the rapid rise of a new SARS-CoV-2 variant and its implications for both immunity and future vaccine updates and design will also be addressed.

CHAPTER 2

Evaluation of Cellular and Serological Responses to Acute SARS-CoV-2 Infection Demonstrate the Functional Importance of the Receptor Binding Domain

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Abstract

The factors that control the development of an effective immune response to the recently emerged SARS-CoV-2 virus are poorly understood. Herein, we provide a cross-sectional analysis of the dynamics of B cell responses to SARS-CoV-2 infection in hospitalized COVID-19 patients. We observe changes in B cell subsets consistent with a robust humoral immune response, including significant expansion of plasmablasts and activated RBD specific memory B cell populations. We observe elevated titers of antibodies to SARS-CoV-2 RBD, full-length spike, and nucleoprotein over the course of infection, with higher levels of RBD-specific IgG correlating with increased serum neutralization. Depletion of RBD-specific antibodies from serum removed a major portion of neutralizing activity in most individuals. Some donors did retain significant residual neutralization activity, suggesting a potential antibody subset targeting non-RBD epitopes. Taken together, these findings are instructive for future vaccine design and monoclonal antibody strategies.

Key Points

- Increased plasmablasts/activated RBD-specific MBCs observed SARS-CoV-2 infection.
- RBD/S/NP titers increase over infection; RBD titers correlate with neutralization**.**
- RBD-specific antibody depletion greatly reduces neutralization in most individuals.

Introduction

The novel coronavirus, SARS-CoV-2, emerged in December 2019 (43) and continues to take an unprecedented toll on the global population with over 2.4 million deaths reported worldwide, a half million of which have occurred in the U.S. alone (47). Two mRNA vaccines (Pfizer-BioNTech and Moderna) are currently approved for emergency use in the United States (279, 280). In addition to these U.S.-approved SARS-CoV-2 vaccines, there are over 200 other vaccine candidates at various developmental stages from preclinical testing to approved usage outside the U.S (281). In order to understand differences in efficacy between vaccine candidates and between immunity developed from vaccination versus natural infection, it is important to continue to explore the characteristics of protective immunity after natural infection. Discoveries concerning the generation, dynamics, and durability of natural immunity may influence discussions and decisions concerning future vaccination development and distribution efforts. Furthermore, a detailed insight into the mechanism of viral neutralization is also essential for both vaccine and monoclonal antibody-based treatment efforts, potentially influencing considerations of necessary antigen targets to achieve effective thresholds of protection.

SARS-CoV-2, a beta-coronavirus (282), shares a high level of homology to SARS-CoV (43), the coronavirus responsible for the 2002-2003 SARS epidemic. These coronaviruses have also been found to share the same host entry receptor, ACE2, which is bound by the receptor binding domain (RBD) on the spike (S) homotrimer that is present on the viral surface (101). RBD is located within the S1 subunit of the protein and appears to be only accessible in the "open" or "up" confirmation of the trimer (101). Given the homology between the two beta-coronaviruses, a predictable relationship between RBD binding and SARS-CoV-2 neutralization exists, and studies by us (163) and others (153, 283, 284) have clearly illustrated a strong

correlation between RBD binding and viral neutralization. Like their SARS-CoV counterparts, antibodies targeting RBD appear to be an integral component of the protective immune response against SARS-CoV-2 (285). In support of this, several antibodies isolated from RBD-specific memory B cells have been characterized and shown to be potent neutralizers of SARS-CoV-2 both *in vitro* (284, 286, 287) and *in vivo* (288). In addition, several groups have shown potent plasmablast responses during acute infection (289), and sizeable RBD- or spike-specific memory B cell responses (284, 286-288, 290) early after infection. Interestingly, potent RBD-specific neutralizing antibodies can be isolated from individuals regardless of the neutralizing serum titer (284) and follicular T cells, a critical part of germinal center reactions, are rarely RBD-specific (290). Given the contribution of antibodies to the neutralization of SARS-CoV-2 *in vivo*, understanding the protective characteristics of the virus-specific B cell responses to SARS-CoV-2 infection remains crucial.

An early expansion of plasmablasts followed by the formation of a circulating antigen-specific memory B cell pool has been reported for numerous acute viral infections, including SARS-CoV-2 (284, 286-290). Previous studies of SARS-CoV and SARS-CoV-2 infection have shown that patients develop severe lymphopenia with significant decreases in T cell numbers (291, 292). In contrast, B cell numbers in these individuals remain unimpacted by infection (291, 292). However, recent evidence from autopsied patients that succumbed to SARS-CoV-2 infection suggests suboptimal germinal center reactions in these patients, which could potentially contribute to short-lived and immature antibody responses to SARS-CoV-2 (196, 289). Additional alterations among B cell subsets have also been reported. For example, expanded atypical memory B cells have been reported in patients with severe COVID-19 (196, 293). A subset most often described in patients with autoimmunity, immunodeficiencies, or chronic viral

infection, these CD27-CD21- B cells are thought to mature independently of germinal center reactions through an extrafollicular pathway (14, 293). The apparent expansion of this subset within severe COVID-19 patients raises questions concerning the nature of their role in the immune response to SARS-CoV-2 and their contribution durable antibody responses, which is a continued concern given several recent reports of SARS-CoV-2 re-infection after only a few months in individuals previously infected with SARS-CoV-2 (294, 295).

Herein, we report a cross-sectional study of the dynamics of human B cell responses during acute SARS-CoV-2 infection. We show that infection induces a potent plasmablast response, and RBD-specific memory B cell responses that correlate with virus-specific serological responses. We also show, using a serum depletion approach, that RBD specific antibodies are the primary driver of viral neutralization in the majority of patients. Interestingly, a subset of the individuals examined had significant portions of their neutralizing response that appeared resistant to RBD depletion, potentially suggesting alternate mechanisms of protection outside the direct inhibition of RBD. These findings have significant implications for ongoing vaccine strategies, as well as for efforts to identify, characterize and deploy preventative and therapeutic monoclonal antibodies against SARS-CoV-2.

Methods

Study cohort

The current study draws on patient samples from hospitalized COVID-19 patients with RT-PCR confirmed SARS-CoV-2 infection at the Emory University Hospital and Emory University Hospital Midtown (n=50). While no specific criteria or demographics were used for enrollment beyond PCR confirmed SARS-CoV-2 infection, all patients were symptomatic at the time of enrollment. Specimens were collected after receiving informed consent, except for 00022371 for which a consent waiver was obtained. The clinical studies from which these samples were obtained was approved by the Emory University Institutional Review Board IRB #00000510, IRB #00045690 and IRB #00022371. For IRB #00000510 and #00045690, informed consent was obtained prior to patient participation. For #00022371, an IRB waiver was obtained allowing the use of discarded samples in the clinical laboratory at the Emory Hospital. The majority of the patients were diagnosed with severe disease (91%) and trended towards being older (median age = 58.5) and male (59%). Further details of the cohort can be found in Supplementary Table 1. Limitations of the cohort include (i) all individuals were hospitalized with the majority diagnosed with severe disease, (ii) a majority of individuals had one or more pre-existing conditions, and (iii) a relatively small sample size.

Sample preparation

Briefly, plasma and PBMC were isolated from peripheral blood collected in CPT tubes from these patients at various times after disease onset (3-57 days post-symptom onset). Briefly, CPT tubes were processed according to manufacturer's protocol, and plasma and PBMCs separated collected separately. PBMCs were treated with ACK lysis buffer (Quality Biological #118-156-

101) for 5 minutes and washed 3 times with PBS with 2% FBS before counting and analysis by flow cytometry. PBMC and plasma were frozen at -80C prior to long-term storage at -80C (plasma) or in liquid nitrogen (PBMC).

Viruses and cells

The infectious clone SARS-CoV-2 (icSARS-CoV-2) and mNG-tagged SARS-CoV-2 (icSARS-CoV-2-mNG) was kindly provided to us and previously described by Dr. Vineet Menachery (UTMB) (296). Briefly, the SARS-CoV-2 virus used was derived from infectious clone 2019-nCOV/USA_WA1/2020 and tagged with a fluorescent reporter gene (mNG) in ORF7 (296). Viral titers were determined by plaque assay on VeroE6 cells (ATCC). VeroE6 cells were cultured in complete DMEM medium consisting of 1x DMEM (Corning Cellgro), 10% FBS, 25 mM HEPES Buffer (Corning Cellgro), 2 mM L-glutamine, 1mM sodium pyruvate, 1x Non-essential Amino Acids, and 1x antibiotics. Viral stocks were titered on VeroE6 cells and stored at -80°C until use.

Flow cytometry

Freshly isolated peripheral blood mononuclear cells were stained first for viability with Live/dead Yellow (ThermoFisher) and then for markers with the following monoclonal antibodies: IgA (IS11-8E10, Miltenyi), IgD (IA6-2, BD), IgG (G18-145, BD), IgM (MHM-88, Biolegend), CD3 (SK7, BD), CD4 (RPA-T4, BD), CD8 (SK1, BD), CD14 (61D3, eBioscience), CD16 (CB16, eBioscience), CD19 (SJ25C1, BD), CD20 (2H7, BD), CD27 (O323, BioLegend or M-T271, BD), CD38 (HB7, BD), and CD71 (CY1G4, BioLegend. Antigen-specific B cells were detected by staining with RBD conjugated to Alexa Fluor 488 (Protein Labeling Kit,

ThermoFisher). RBD was conjugated according to manufacturer's instructions, with the following changes: protein was labeled at a concentration of 1mg/mL, and incubated for 30 minutes without the addition of bicarbonate. After staining, PBMCs were washed and then fixed for 30 minutes using 2% paraformaldehyde (ThermoFisher). Data were acquired on a BD FACSymphony A5 and analyzed using FlowJo 10.7.1 (BD).

ELISA

ELISAs were conducted as we have previously described (163). Recombinant RBD for this assay was generated as previously described (163). Briefly, recombinant RBD derived from SARS-CoV-2, Wuhan-Hu-1 (GenPept:QHD43416) was cloned, expressed in an Expi293F cell system, and purified on HisTALON Superflow Cartridges (163). Recombinant RBD, recombinant monomeric spike (obtained from the CDC), or nucleoprotein (Sinobiological, # 40588-V08B) were coated overnight at 4ºC on Maxisorb plates at 0.5 (NP) and 1 (RBD, spike) µg/mL in Dulbecco's phosphate-buffered saline (DPBS). After blocking for 2 h with 1% bovine serum albumin (BSA) in PBS containing 0.05%Tween 20 (PBS-T), serially diluted serum samples were added and incubated for 90 minutes. The bound antibodies were detected using goat anti-human isotype specific secondary antibodies conjugated to horseradish peroxidase (HRP) that were added for 60 minutes (Jackson ImmunoResearch, anti-IgG Cat#109-036-098, anti-IgM Cat#109-036-129, anti-IgA Cat#109-036-011). Plates were developed with 0.4 mg/mL o-phenylenediamine dihydrochloride (OPD) diluted in phosphate-citrate buffer pH 5.0 containing 0.012% H_2O_2. The reaction was stopped with 1M hydrochloric acid and the absorbance was measured at 490 nm using a spectrophotometer (BioRad). Unless noted, plates were washed 3 times with PBS-T between each step. Endpoint titers were interpolated based on a

sigmoidal 4-parameter logistic where X is concentration with the baseline value for each isotype/antigen pair derived from the average plus three times the standard deviation of pre-pandemic negative control samples (n=20).

Focus Reduction Neutralization Titer assay

COVID-19 patient or healthy control plasma were incubated at 56°C for 30 min and manually diluted in duplicate in serum-free Dulbecco's modified media and incubated with 750-1000 focus-forming units of either icSARS-CoV-2 or SARS-CoV-2-mNG virus at 37º C for 1 hour. The virus/serum mixture was added to VeroE6 cell monolayers seeded in 96-well clear or blackout plates and incubated at 37º C for 1 hour. Post incubation, the inoculum was removed and replaced with pre-warmed complete DMEM containing 0.85% methylcellulose. Plates were incubated at 37º C for 24 hours. After 24 hours, the methylcellulose overlay was removed, cells were washed three times with phosphate-buffered saline (PBS), and fixed with 2% paraformaldehyde (PFA) in PBS for 30 minutes at room temperature. For the FRNT assay, plates were washed twice with 1x PBS and 100 µl of permeabilization buffer (0.1% BSA-Saponin in PBS) (Sigma Aldrich), was added to the fixated Vero cell monolayer for 20 minutes. Cells were incubated with an anti-SARS-CoV spike protein primary antibody conjugated to biotin (CR3022-biotin) for 1 hour at room temperature, then with avidin-HRP conjugated secondary antibody for 1 hour at room temperature. Foci were visualized using True Blue HRP substrate and imaged on an ELISPOT reader (CTL). For the FRNT-mNG assay, the 2% PFA is removed and washed twice with PBS. The foci were visualized using an ELISPOT reader (CTL ImmunoSpot S6 Universal Analyzer) under a FITC channel and enumerated using Viridot. The neutralization titers were calculated as follows: 1 - (ratio of the mean number of foci in the

presence of sera and foci at the highest dilution of respective sera sample). Each specimen is tested in two independent assays performed at different times. The FRNT-mNG$_{50}$ titers were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 8.4.3. Samples with an FRNT$_{50}$ value that was below the limit of detection, are plotted at 10. For these samples, this value was used in fold reduction calculations.

Depletion of RBD specific serum antibody

RBD binding antibodies in patient sera were depleted using RBD-coupled paramagnetic beads. Recombinant SARS-CoV-2 RBD was covalently attached to paramagnetic M-270 epoxy Dynabeads using the "Dynabeads Antibody Coupling Kit" (ThermoFisher Scientific # 14311D) according to manufacturer's instructions for labeling 60 mg of beads. Beads were prepared using 30 µg of RBD per mg of beads. After coupling, beads were suspended at a concentration of 10 mg/mL in buffer SB containing 0.02% (w/v) sodium azide for up to two weeks at 4ºC. Immediately before use, RBD-coupled beads were washed once for 5 minutes in PBS with 0.1% BSA and then resuspended in DPBS at a concentration of 30 mg/mL. Patient sera were added to beads at a ratio of 1:10 (v/v) and gently mixed for 1 hour at ambient temperature using a rotating mixer. Depleted sera were separated from beads with a magnet tube rack and transferred to a fresh tube that contained RBD coupled beads equal in amount to the first depletion, which had been separated from the storage solution. Samples were incubated again for 1 hour at ambient temperature and then magnetically separated from beads yielding RBD-depleted sera diluted 1 to 10 in DPBS. Samples were aliquoted and stored at -80ºC prior to use in binding assays or neutralization assays as described above. Endpoint binding titers for this assay were interpolated based on a sigmoidal 4-parameter logistic where X is concentration using 3x background as the

baseline value. Neutralization titers were calculated as previously stated. Percent reduction was then calculated from the fold-change between the pre-depletion and post-depletion samples.

Statistics

Data were analyzed using GraphPad Prism 8.4.3. A one-way ANOVA Brown-Forsythe test or Holm-Sidak multiple-T test, as appropriate for all comparisons of cell populations and antibody titers between groups. Pearson correlation coefficients and linear regressions were applied as appropriate.

Results

Highly expanded plasmablasts and reduced memory B cell frequencies in peripheral blood of hospitalized COVID-19 patients.

To define the dynamics of human B cell responses during acute SARS-CoV-2 infection, we assessed CD19+ cell subsets in a cross-sectional study of 46 hospitalized patients, sampled at timepoints ranging from 3 to 57 days post symptom onset (DpSO), compared to 8 healthy controls samples collected during the study period and confirmed to be SARS-CoV-2 negative by serology (Figure 1). Six of the acutely infected patients were sampled at least twice (Supplementary Table 1). We found that the overall B cell compartment (CD19+ cells) in peripheral blood was significantly increased in the second (19.1±7.54) and third (15.1±6.69) weeks after symptom onset as compared to healthy controls (9.16±2.67) ($p<0.001$, $p=0.017$). This increase is likely due to both plasmablast expansion as well as a loss of peripheral CD3+ T cells (Supplemental Fig 1), as has been previously reported.[297] Focusing on antigen-experienced B cell subsets, we analyzed both infection-induced plasmablasts and total memory B cells (MBC). We found highly expanded plasmablast responses in the majority of COVID-19 patients, rising early after symptom (<d7) onset (5.0±1.7, $p=0.016$), peaking at 8-14 DpSO (19.5±17.6, $p<0.001$), and remaining significantly increased 15-21 DpSO (14.5±14.5 $p=0.006$), as compared to healthy controls (1.3±0.6). In contrast, classical MBCs (CD27+/IgD- B cells) were significantly reduced, falling before 7 DpSO (HC=23.2±7.2, CVD=9.4±3.3, $p=0.003$) and remaining low in patients at >21 days of illness (13.4±5.8, $p=0.044$). CD27- MBCs were not significantly reduced in frequency at any timepoint. Additionally, unswitched memory B cells (CD27+/IgD+) were dramatically reduced ≤7 DpSO (2.6±0.9) compared to controls (14.2±6.9)

(p=0.008) and remained low in patients hospitalized >21 DpSO (3.2±2.2, p=0.008). Unswitched MBC are known to exhibit reactivity similar to naïve B cells, and are able to rapidly respond to antigen (14). The significant loss observed may be in part due to differentiation into plasmablasts, but this issue requires further study. However, both CD27+ and CD27- switched MBCs remained low after 21 DpSO, when plasmablasts were no longer significantly expanded, at least in peripheral blood. These data show an early expansion of plasmablasts that is reminiscent of other serious viral infections, such as H1N1 influenza (298), dengue (188) and Ebola (299) infection, and is likely responsible for the early SARS-CoV-2-specific antibody responses seen in these patients.

RBD-specific memory cells appear 8-14 days after symptom onset.

To further assess the dynamics of B cell responses to SARS-CoV-2, we analyzed antigen-specific memory B cell responses by flow cytometry, using fluorescently-labeled RBD as a probe (Figure 2A). We observed a significant expansion of RBD-specific switched MBC at 8-14 DpSO, corresponding to 0.57±0.53% of the overall MBC population, compared to the negligible background of 0.07±0.02% in the healthy controls (p=0.005) (Figure 2B). The mean frequency of RBD-specific MBCs continued to increase 15-21 DpSO (0.63±0.33%, p=0.004), and then plateaued >21 DpSO (0.63±0.37, p=0.027). Notably, in some patients, a proportion of the RBD-specific switched MBCs did not express CD27 (Supplemental Fig 2). In fact, overall 31.6±19.5% of the RBD+ cells were CD27-, and in one participant reached as high as 74%. This observation could be connected to the loss of CD27+ switched MBCs, as the frequency of CD27 expression did not differ between RBD-specific and non-specific MBCs, or a sign that some RBD+ MBCs

are generated in an early extrafollicular or T-independent manner (300). Therefore, we have reported RBD-specificity as a function of total switched MBCs (Figure 2B).

The RBD+ MBC in COVID-19 patients are primarily of the IgG isotype (Figure 2C/D). RBD-specific IgM+ MBC were only significantly expanded at 8-14 DpSO (0.08±0.11) compared to healthy controls (0.005±0.005) (p=0.04) and responses at that time were highly variable. IgA+ MBCs were also only significantly expanded at 8-14 DpSO (0.15±0.12) compared to healthy controls (0.01±0.01) (p=0.002). Though a subset of patients did have measurable RBD+ IgA+ MBCs at later timepoints, other patients did not seem to mount strong IgA+ MBC responses. In contrast, IgG+ RBD-specific MBCs were significantly expanded starting at 8-14 DpSO (0.26±0.27, p=0.02) through 15-21 DpSO (0.29±0.20, p=0.02). By 21 DpSO or later all donors were positive for IgG+ RBD-specific cells (0.45±0.23, p=0.01) (Figure 2D). Our data show that RBD-specific switched MBC arise by the second week of infection, and highlight that focusing on only CD27+ memory may exclude a sizeable percentage of the RBD-specific memory response.

RBD-specific MBCs upregulate the activation marker CD71.

Recently, activated memory B cells (ABCs) have been shown to be an important subset in several diseases, such as Ebola and influenza (299). Therefore, we assessed the expression of CD71 on the memory B cells of healthy controls and patients with acute SARS CoV-2 infection, and further compared the CD71 expression of non-RBD and RBD-binding memory B cells during disease progression (Figure 2E-F). MBC obtained from healthy controls had low

frequencies of CD71+ (6.8±1.9). In contrast, the frequency of activated switched MBCs in COVID-19 patients were significantly higher (14.3±8.0) ($p<0.001$). The RBD-specific switched MBCs express CD71 at markedly higher frequencies (42.5±21.3) than non-RBD-specific MBCs. This difference was not only significant compared to healthy control MBC ($p<0.001$) but was also significantly increased relative to non-RBD-specific MBC from the same patients ($p<0.001$). The difference between RBD-specific and non-specific MBC was most apparent between days 8 and 14 (RBD=44.8±16.1 vs non-RBD=13.7±7.29, $p<0.001$) and days 14 and 21 (RBD=27.2±20.1 vs non-RBD=17.7±10.0, $p=0.001$). These data show that not only are RBD-specific MBCs are present early in the course of COVID-19, but that these B cells are an active part of the ongoing immune response.

Circulating IgG and IgA titers against RBD, S, and NP antigens peak 3 weeks post-symptom onset.

To determine the dynamics of antibody responses during infection, we measured circulating antibody titers against SARS-CoV-2 antigens by ELISA in the 46 individuals analyzed above, using recombinant RBD, monomeric spike (S), or nucleoprotein (NP). In agreement with previous reports showing that seroconversion against SARS-CoV-2 and other coronaviruses occurs within two weeks post-symptom onset (153, 282, 283), all but two individuals in the cohort had positive IgG and IgA titers against all three antigens by two weeks post-symptom onset. IgG and IgA titers against all antigens trended with DpSO with significant increases in antibody titer observed between the first, second, and third weeks post-symptom onset with the NP-specific IgG serum fraction (Figure 3A). Between 8-14 DpSO, 93% (25/27), 93%, (25/27),

and 96% (26/27) of individuals had positive IgG titers towards RBD, S, and NP, respectively, as compared to 88% (24/27), 78% (21/27), and 81% (22/27) of individuals with positive IgA titers (Figure 3A, Supplementary Table 1). We also note that, while IgG titers against all antigens remain robust in individuals greater than a month post-symptom onset, IgA titers tended to decrease in the individuals sampled one-month post onset as compared to the early timepoints (Figure 3A). Thus, the antibody responses to SARS-COV-2 infection were dominated by IgG, even early after infection, illustrating that isotype switching occurs rapidly during the acute infection, with lower level responses of the IgM and IgA isotypes also detectable in most donors. Almost all of the acutely infected hospitalized patients had detectable SARS-CoV-2 neutralizing antibody responses (Figure 3B) with an average reciprocal titer of 568 and a range from 23 to 2205 (Supplementary Table 1). These responses displayed a strong correlation with RBD-specific IgG antibody titers, as we have previously shown (Figure 3B) (163). Although weaker than the correlation with RBD-specific IgG titers, neutralization titers also had a positive correlation with anti-S IgG titers (Figure 3B). Finally, NP-specific IgG titers correlated quite poorly with SARS-CoV-2 neutralization (Figure 3B). Overall, this data illustrates the occurrence of a rapid and robust antibody response to multiple SARS-CoV-2 antigens in individuals with severe COVID-19.

The RBD-specific serum fraction is responsible for neutralizing activity in a majority, but not all, hospitalized COVID-19 patients.

As has been previously reported, circulating titers of RBD-specific IgG correlated with time after disease onset (163) (Supplemental Fig 3) and with serum neutralizing potency (Figure 3B).

Given differences in time of sampling between patients and limited clinical data, we were unable to correlate metrics of disease severity or resolution within this cohort to RBD-specific titers (Supplementary Table 1). To quantify the overall contribution of RBD-specific antibodies to SARS-CoV-2 viral neutralization, we depleted RBD binding antibodies from serum samples collected from a randomly selected subset of infected patients. To assess the effectiveness of the depletion, we determined the endpoint RBD binding titer for paired pre- and post-depletion serum samples. We found that all samples were efficiently depleted with an average percent reduction of RBD specific IgG of 98% (Figure 4B). Irrespective of the initial titer (Figure 4A/B), it is important to note that a subset of individuals had post-depletion titers that dropped below the limit of detection (Figure 4A). For these individuals, the fold reduction was estimated using half the limit of detection as a baseline value (10) and therefore may be greater than what was measurable in this assay. The pre- and post-depletion serum samples from each individual were then analyzed using a viral neutralization assay. Pre-depletion, reciprocal neutralization titers ranged from 154 to 10,270 with a median titer of 973 (Figure 4C). Post-depletion, 13 individuals had titers above baseline, and the remaining individuals had titers below the limit of detection (Figure 4C). The neutralization potency of depleted serum samples was markedly reduced in the majority of individuals assayed relative to pre-depletion control samples. Specifically, 13 of 19 of serum samples had a greater than 80% reduction in the viral neutralization titers as a consequence of depleting the RBD binding antibodies (Figure 4C&D). These results provide evidence that epitopes within the RBD are the main target of antibody-mediated viral neutralization in these individuals. In the remaining 6 individuals, 4 had >65% reduction in neutralization titer, and the remaining two individuals had 49.7% and 30.3% reductions in neutralization, respectively (Figure 4C&D). This observation indicates that these donors retained

more than 30% of their neutralization activity despite RBD depletion (Figure 4D). This result indicates that over one third of the neutralizing activity in these individuals may be due to antibodies that do not bind directly to the RBD region of spike or that bind to a confirmation of RBD that is not preserved in its recombinant form. Taken together, this analysis shows that while the majority of neutralizing antibodies are RBD specific, some individuals may generate neutralizing responses that target non-RBD epitopes. These antibodies may represent an important class of immunoglobulins that could act in synergy with clinically relevant RBD-specific neutralizing antibodies or enhance protection to other coronaviruses and SARS-CoV-2 RBD escape variants.

Discussion

Important components of the humoral response to viral infection include not only a rapid expansion of antibody secreting cells (ASCs) to boost circulating serum titers towards the invading pathogen but also the formation of an antigen-specific memory B cell pool responsible for long-lasting protection. While multiple groups have described strong B cell responses in SARS-CoV-2 patients, and RBD specific memory B cells encoding neutralizing antibodies at convalescence, the dynamics of these responses have not been well characterized, either cross-sectionally or longitudinally. Furthermore, several recent reports (196, 293) have described "dysregulated" B cell responses during severe SARS-CoV-2 infection, suggesting mechanisms that could lead to ineffective and short-lived antibody responses, as in the case of chronic viral infections such as HIV (301) and HCV (302). A failure to develop or a later loss of germinal center structures within the lymph nodes of deceased COVID-19 patients (289) and the abundance of several extrafollicular B cell populations in severe COVID-19 patients, normally observed in autoimmune individuals (such as double negative (DN) B cells) (196), have suggested this dysregulation. In our cohort of acutely infected SARS-CoV-2 patients, we found robust infection-induced plasmablast responses and the development of RBD-specific MBCs, which exhibited greater activation than their non-RBD-specific counterparts. Taken together, this data provides evidence for robust and functional humoral response to SARS-CoV-2 infection even in the face of severe disease. However, we also observe that, within the RBD-specific MBC compartment, a significant fraction of the cells are negative for CD27, a population that has been previously described to have an extrafollicular origin (14). This finding is line with previous reports (196, 289, 293) that SARS-CoV-2 infection generates an extrafollicular response and raises questions as to the contribution of these extrafollicular subsets to the robustness and

durability of the immune response against SARS-CoV-2. In addition, we observe a significant decrease of the unswitched MBC population in infected individuals, which could also be suggestive of immune dysregulation. These findings clearly highlight the heterogeneity of COVID-19 as a disease and the continued need to dissect the cellular response to SARS-CoV-2 infection.

In the case of previously studied coronaviruses, both pandemic and endemic species, seroconversion has been reported to take place within 2-3 weeks from the time of infection (129, 303, 304). It has now been well-documented in cohorts containing both mild and severe cases of COVID-19 that, on average, seroconversion takes place two weeks post-infection (153, 283). Our serological analysis of a cohort of severe COVID-19 patients supports the findings of previously published reports with the majority of individuals exhibiting positive titers against multiple SARS-CoV-2 antigens by two weeks post symptom onset. This analysis suggests that, even in individuals with severe COVID-19, the humoral response to SARS-CoV-2 remains functional. In fact, as has been previously reported (283, 284), individuals with greater disease severity tend to have higher levels of RBD-binding antibodies in circulation, suggesting a robust humoral response to infection. While this trend could be potentially due to later seroconversion in these individuals, i.e. a delayed antibody response, previous studies of SARS-CoV patients demonstrate that patients with both earlier seroconversion and, in some cases, higher antibody titers were more likely to experience severe disease (305, 306). The data presented herein supports a model in which neutralizing antibodies may be insufficient for mitigating disease progression and pathology in certain individuals. Critical questions remain as to why circulating antibody responses observed in our cohort were unable to prevent severe disease given that the serum antibodies were able to bind effectively to multiple viral antigens and potently neutralize

the virus *in vitro*. Thus, the contribution and functional role of the humoral response in severe SARS-CoV-2 infection *in vivo* still needs to be elucidated.

We have previously reported a highly significant correlation between serum neutralizing potency *in vitro* and RBD binding titers (163), as have others (283, 284). However, recent investigations into both the cellular and serological aspects of the B cell response to SARS-CoV-2 infection have begun to raise questions about the contribution of antibodies derived against additional antigenic targets (290). While the strongest correlation within our cohort is undoubtably between anti-RBD IgG titers and serum neutralization, significant correlations can also be found between full length S and NP antibody titers and serum neutralization. Despite the significant body of evidence now exists that supports the neutralizing potential of RBD-specific antibodies (172), it is possible that antibodies targeting epitopes outside of the RBD epitopes could also contribute to neutralization potency. To provide quantitative evidence for the role of RBD-specific antibodies in circulating serum neutralization, we employed a strategy similar to that previously published by He, et al after the SARS pandemic in 2002-2003 (148). We show that depletion of the RBD-specific serum fraction reduced the neutralizing potency of the remaining serum antibody by greater than 80% in 13 out 19 individual serum samples tested. Interestingly, the percentage of RBD-specific B cells observed within the cohort represented an exceedingly small percentage of the overall MBC population. The contrast between the small percentage of RBD-specific MBCs observed and their potent contribution to the neutralizing activity is echoed by the findings of Rogers et al., where the percentage of RBD specific antibodies derived from spike-specific MBCs was minimal and yet the RBD-specific antibodies contributed an equal number of neutralizing antibodies as their non-RBD counterparts (307). In addition, this group also found that the non-RBD antibodies had lower neutralization than their

RBD-specific counterparts and failed to provide protection in an *in vivo* small animal model (307). In contrast, a subset of the donors we analyzed showed a significant residual activity after RBD depletion, such that greater than 30% of the neutralization activity remained after depletion. There could be a number of explanations for this difference. It is possible that non-RBD antibodies are most potent in a synergistic environment in which antibodies against multiple epitopes or antigens act together to elicit neutralizing responses – this hypothesis would explain why neutralization effects observed in serum are not seen when testing monoclonal antibodies. Alternatively, these donors may have initiated a response that produced potently neutralizing antibodies to non-RBD epitopes. Analysis of monoclonal antibodies derived from these donors is currently ongoing. Thus, in conclusion, while our study shows that the majority of neutralizing activity in circulating serum is driven by RBD-specific antibodies, questions remain concerning the importance and combinatorial potency of non-RBD antibodies *in vivo*. This finding has potential implications for vaccine design, as it appears that generation of antibodies targeting solely SARS-CoV-2 RBD are sufficient for viral neutralization in the majority of individuals assayed. Thus, vaccines containing RBD rather than FL spike or whole virus would seem likely provide sufficient, if not greater, elicitation of SARS-CoV-2 neutralizing antibodies. It was also found that a subset of individuals possess neutralizing antibodies targeting potentially non-RBD epitopes, which could lead to the discovery of potent neutralization targets outside the RBD. However, further investigation is necessary to ascertain the targets of the neutralizing antibodies, as confirmation of RBD can be critical for the function of specific antibody subsets and thus, we cannot confirm that these antibodies do not bind to RBD in some form. Taken together, these findings serve as a platform for further exploration of the immune

response to SARS-CoV-2 and will be instructive for current vaccine design and development and optimization of prophylactic and therapeutic strategies based on monoclonal antibodies

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Author Contributions

G.M., L.E.N., and R.C.K contributed to the acquisition, analysis, and interpretation of data and writing the manuscript. V.V.E., L.L., and K.F. performed *in vitro* neutralization experiments. P.S. and V.D.M. provide the viral clones used for *in vitro* neutralization assays. S.E. served as the principal investigator of the clinical protocol for acquisition of patient samples. E.M.S., A.K.., and N.M. provided clinical support for the study and contributed to sample collection and

analysis. N.R., R.A., M.S.S., and J.W. contributed to the conception and design of the work and the writing and approval of the final manuscript.

Declaration of Interests

E.J.A has received personal fees from AbbVie, Pfizer, and Sanofi Pasteur for consulting, and his institution receives funds to conduct clinical research unrelated to this manuscript from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Novavax, Sanofi-Pasteur, Janssen, and Micron. He also serves on a safety monitoring board for Kentucky BioProcessing, Inc.

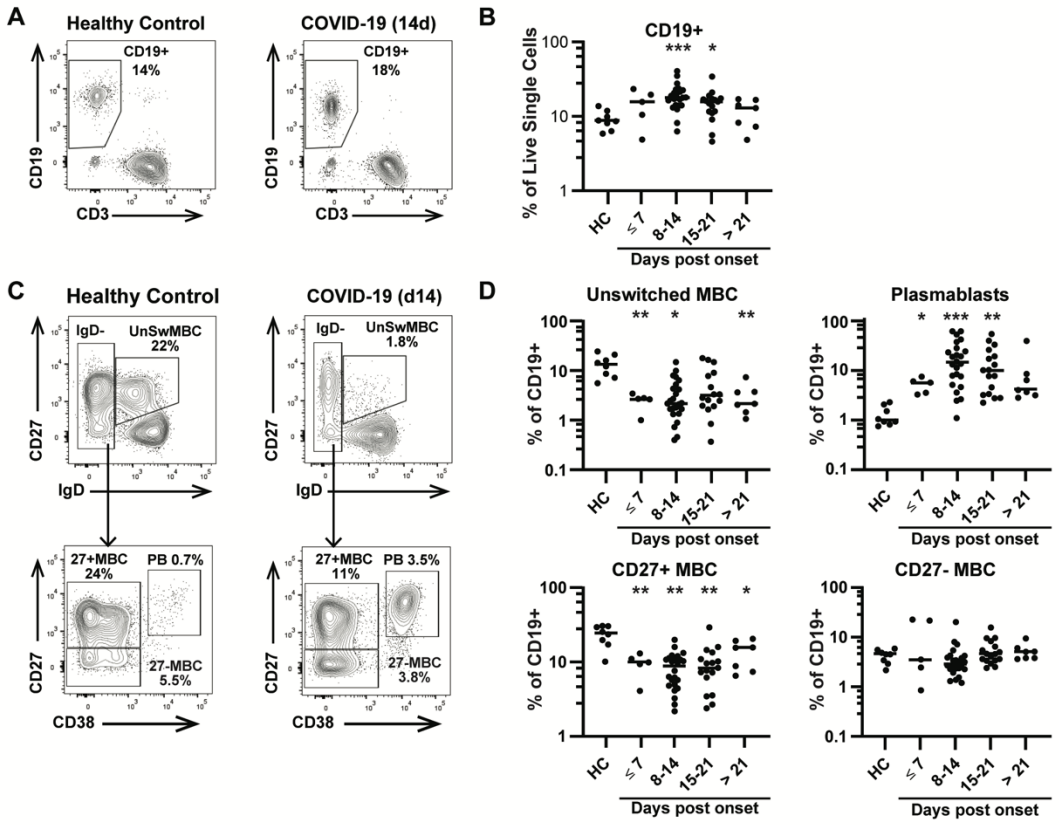


Figure 1. Acute COVID-19 patients exhibit loss of circulating memory B cells and expansion of plasmablasts. A) CD19+ B cells are identified from live single $CD14^{-}CD16^{-}$ cells in a healthy control (left) or COVID-19 (right) participant. B) Percentage of CD19+ B cells are shown for healthy controls (n=8) or hospitalized COVID-19 patients (n=46) over time, measured as days post symptom onset. Six patients contributed more than one timepoint. C) CD19+ B cells are further subsetted as unswitched memory B cells (MBC), isotype-switched CD27+ MBC and CD27- MBC, and plasmablasts (PB). Unswitched MBC are identified as CD27+IgD+, while MBC and PB are IgD- and then separated by CD27 and CD38 expression. D) Unswitched MBC, CD27+ MBC, CD27- MBC and plasmablasts are shown as % of CD19+ cells in healthy controls and COVID-19 patients. Significance is calculated by Brown-Forsythe ANOVA test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

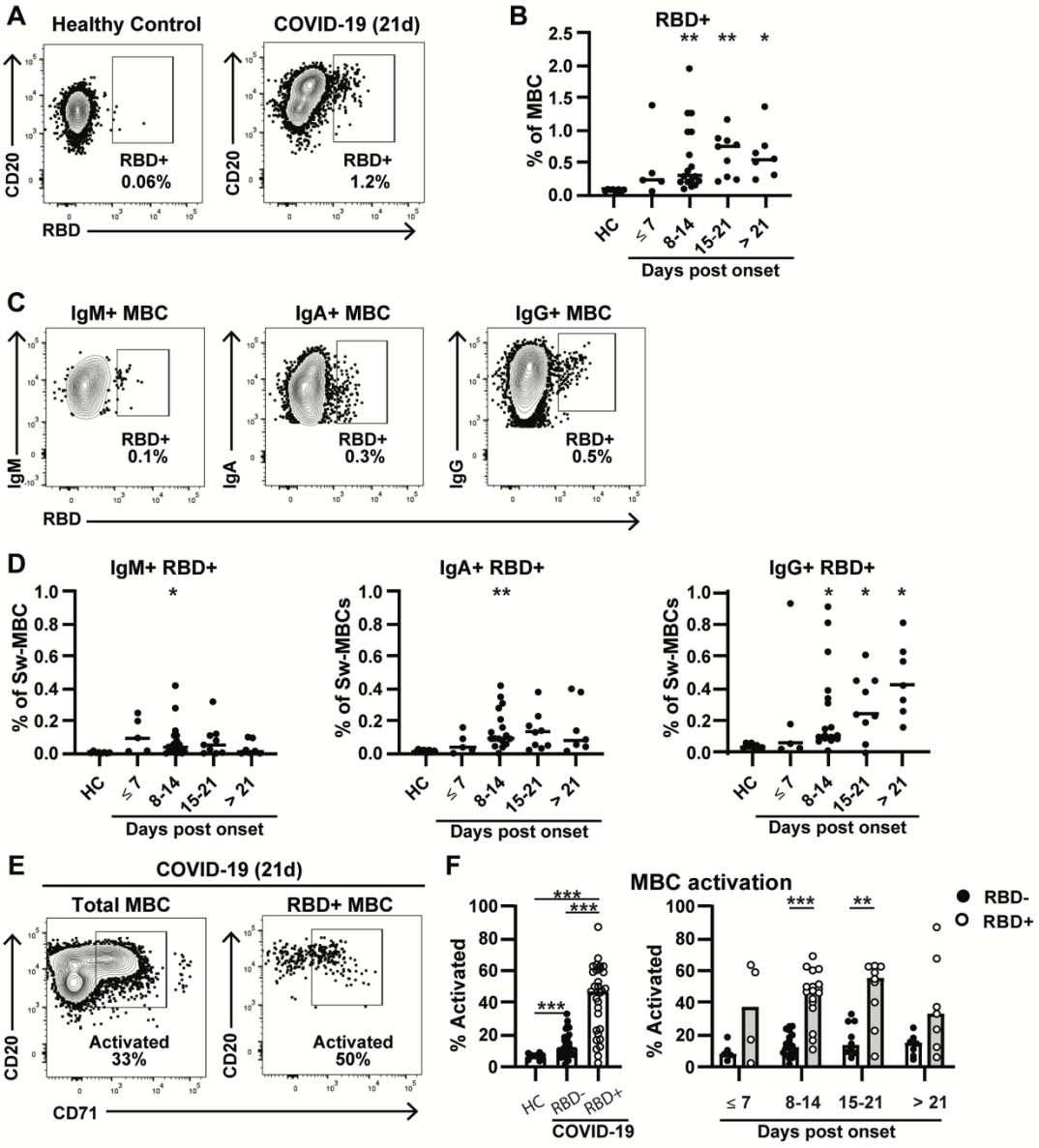

A
Healthy Control
COVID-19 (21d)
CD20
RBD
RBD+
0.06%
RBD+
1.2%
B
RBD+
% of MBC
HC
≤ 7
8-14
15-21
> 21
Days post onset
C
IgM+ MBC
IgA+ MBC
IgG+ MBC
IgM
IgA
IgG
RBD+
0.1%
RBD+
0.3%
RBD+
0.5%
RBD
D
IgM+ RBD+
IgA+ RBD+
IgG+ RBD+
% of Sw-MBC
% of Sw-MBCs
Days post onset
E
COVID-19 (21d)
Total MBC
RBD+ MBC
CD20
CD71
Activated
33%
Activated
50%
F
MBC activation
% Activated
HC
RBD-
RBD+
COVID-19
≤ 7
8-14
15-21
> 21
Days post onset
RBD-
RBD+

Figure 2. RBD-specific memory B cells expand rapidly and exhibit high levels of activation in COVID-19 patients. A) RBD-specific memory B cells (CD19+CD20+IgD-CD38-) are shown for a healthy control (left) or COVID-19 patient (right). B) RBD+ MBCs are shown as a percentage of total MBCs for healthy controls (n=8) and COVID-19 patients (n=34) over time. Four COVID-19 patients contribute repeat timepoints. C) Gating is shown for IgM+ (left), IgA+ (middle), and IgG+ (right) RBD+ MBCs in the COVID-19 patient shown in (A). Percentages shown are % of total MBC. D) RBD+ MBCs are shown as in (B), split into IgM+ (left), IgA+ (middle) and IgG+ (right). E) Activated B cells, gated by CD71 expression, for both total (left) and RBD+ (right) MBC. F, left) Total activation in healthy controls (n=8) and COVID-19 patients RBD- MBC (n=33) or RBD+ MBC (n=30). F, right) A comparison of activation over time between RBD- and RBD+ MBC. Significance is calculated by Brown-Forsythe ANOVA test (B, D, F left) or Holm-Sidak multiple T test (F right). *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

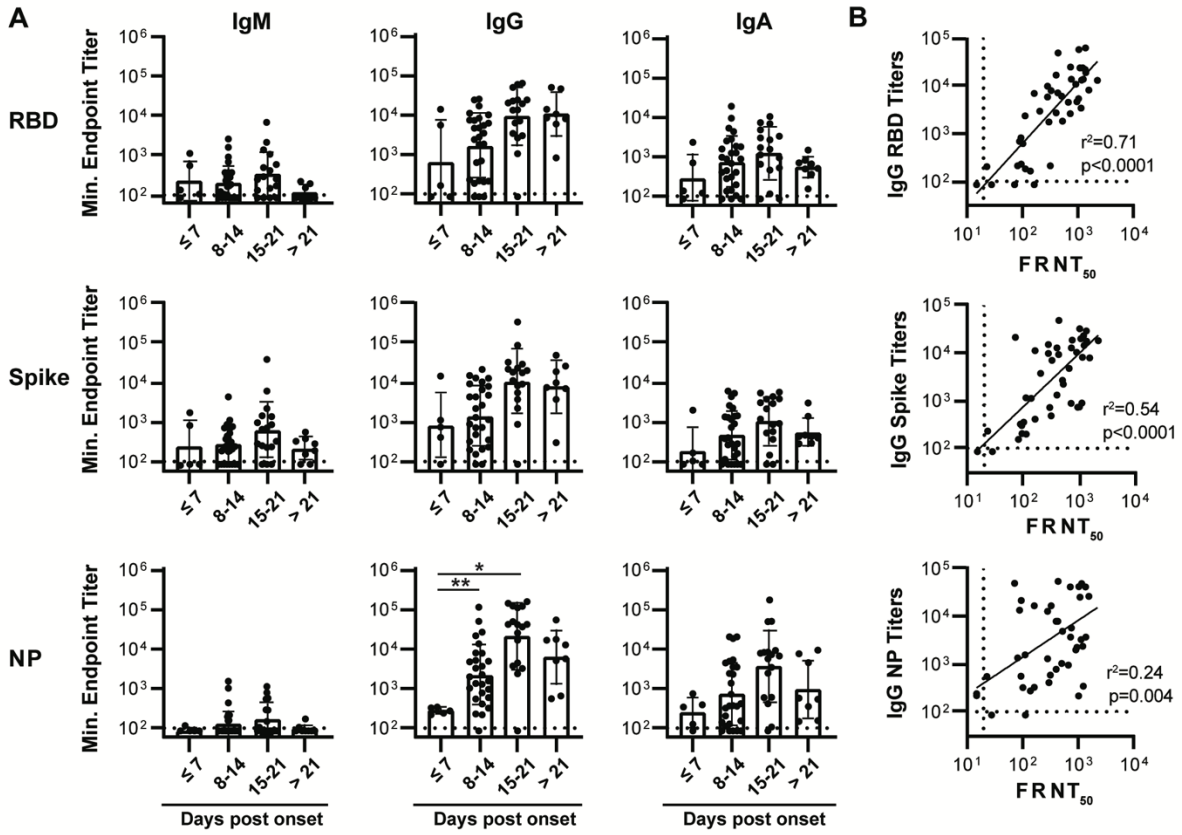


Figure 3. RBD-binding fraction of patient serum antibody strongly correlates with neutralization capacity. (A) ELISA endpoint titers for serum binding against SARS-CoV-2 receptor binding domain (RBD), spike (S), and nucleoprotein (NP) recombinant protein from a cohort of acutely infected individuals (n=46). Significance is calculated by Brown-Forsythe ANOVA test. *p≤0.05, **p≤0.01 (B) IgG binding titers against RBD, spike, and NP correlated with SARS-CoV-2 serum neutralization activity. The coefficient of determination (r2) is reported following linear regression analysis.

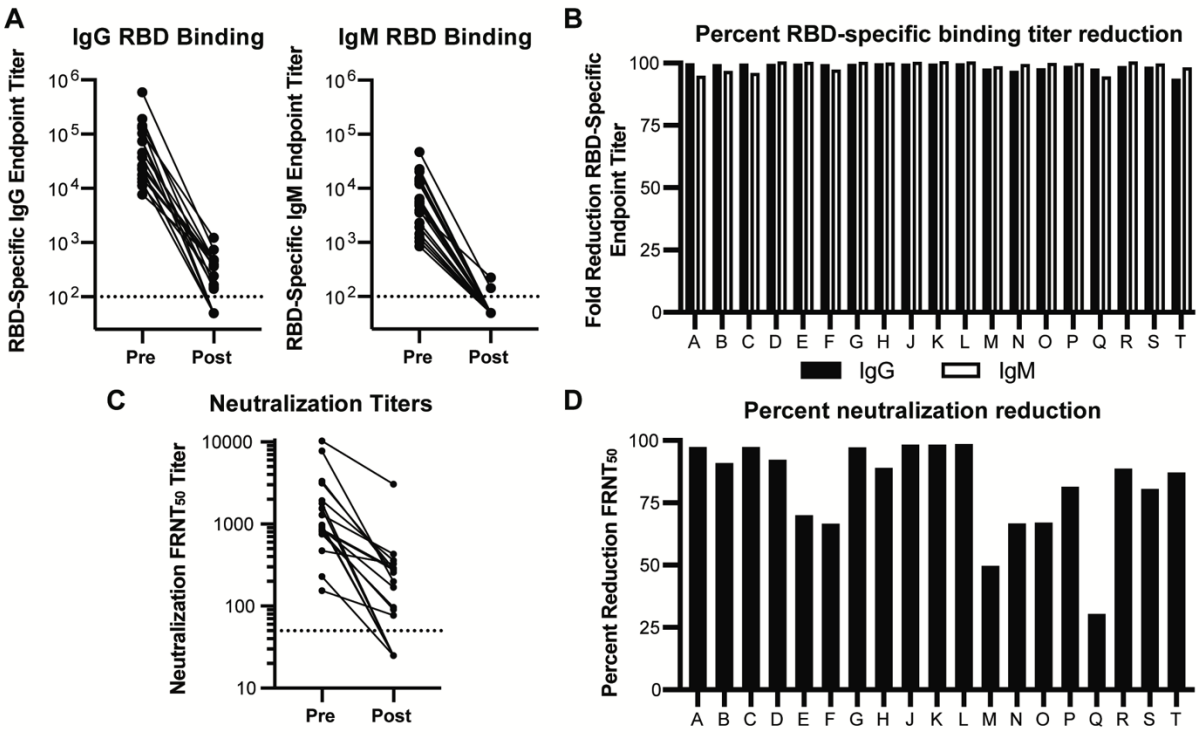
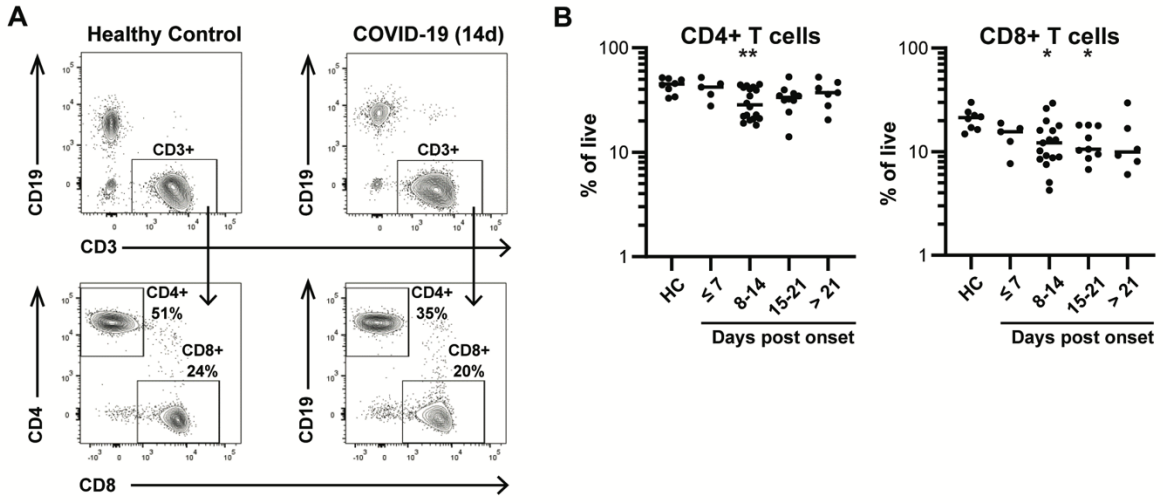
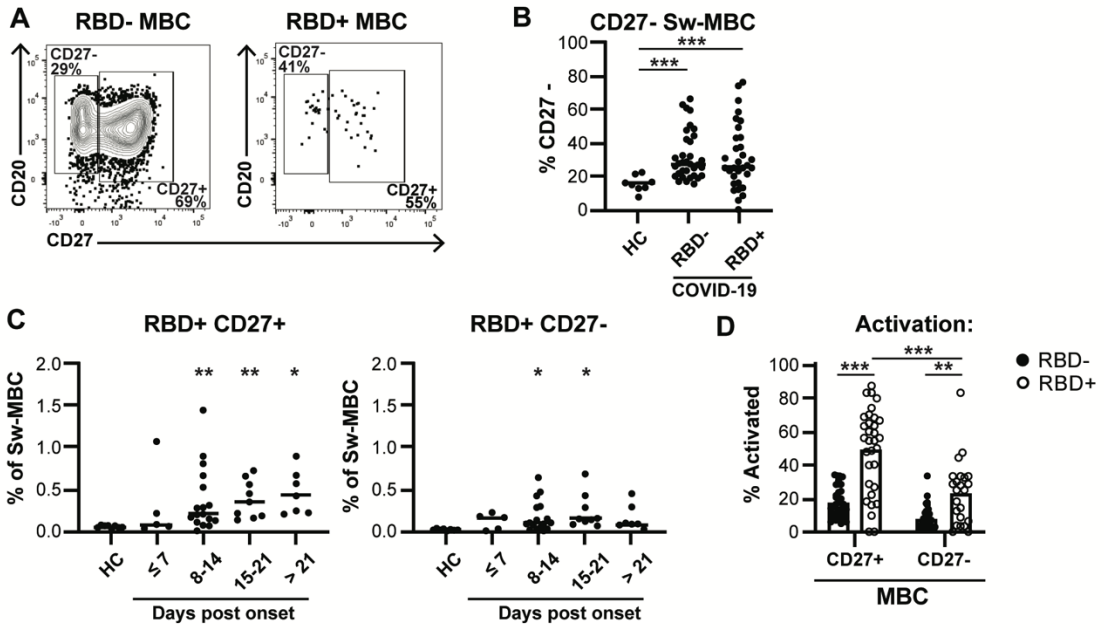


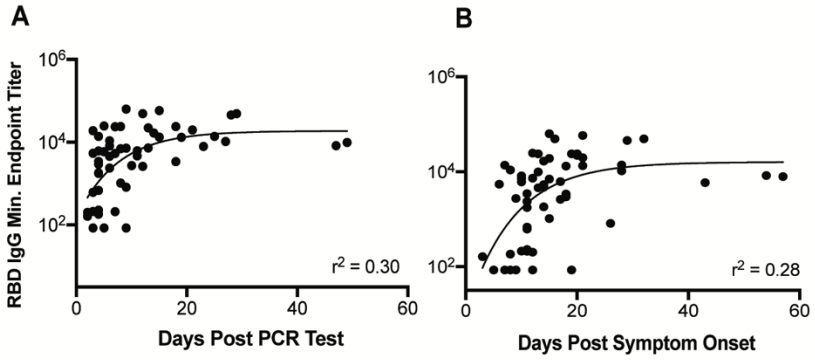
Figure 4. SARS-CoV-2 viral neutralization activity is mediated by RBD specific antibody in a majority of COVID-19 patients. (A) ELISA endpoint titers for SARS-CoV-2 receptor binding domain (RBD) specific IgG and IgM in sera from 19 acute COVID-19 patients before (Pre) and after (Post) depletion of RBD binding antibodies. (B) For each patient, bars represent the percent reduction in serum IgG (black) or IgM (white) RBD binding endpoint titers relative to pre-depleted samples. (C) Serum neutralization activity against SARS-CoV-2 before and after depleting RBD binding antibodies. Values represent the $FRNT_{50}$ titer. (D) For each donor, the effect of reducing RBD binding serum antibodies on the viral neutralization titer is expressed as percent reduction in the $FRNT_{50}$ value relative to pre-depleted samples.



Supplemental Figure 1. CD4+ and CD8+ T cells decreased in COVID-19 patients. A) CD3+ T cells gated by expression of CD4 and CD8 in a healthy control (left) vs a COVID-19 patient 14 DpSO. B) Percentage of CD4+ (left) and CD8+ (right) cells are shown for healthy controls (n=8) or hospitalized COVID-19 patients (n=47) over time, measured as days post symptom onset. Six patients contributed more than one timepoint. Significance is calculated by Brown-Forsythe ANOVA test or Holm-Sidak multiple T test. *p≤0.05, ** p≤0.01



Supplemental Figure 2. RBD-specific MBCs are found in both the CD27+ and CD27- subsets. A) RBD-binding (right) and non-binding (left) MBCs are gated by expression of CD27. B) The percent of CD27-negative healthy control (n=8), non-RBD-specific (n=33) and RBD-specific (n=30) MBCs. C) RBD-specific CD27+ (left) or CD27- (right) MBCs are shown as the percent of total switched-MBC for healthy controls (n=8) and COVID-19 patients (n=34) over time. D) Activation of RBD-specific and non-specific MBCs in COVID-19 patients, divided by CD27 expression. Significance is calculated by Brown-Forsythe ANOVA test. *p≤0.05, **p≤0.01, ***p≤0.001



Supplemental Figure 3. RBD-specific IgG titers correlate with time from infection. Days post infection as measured by either (A) positive PCR test or (B) self-reported days post-symptom onset plotted against circulating RBD-specific IgG. R2 values are calculated from an exponential plateau best-fit model in Prism.

PTID	Sex	Age	Disease Severity	Days From Positive PCR Test	Days From Symptom Onset	Total Days in Hospital	Fatal	RBD			Full Spike			Nucleoprotein			FRNT50
								IgM	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	
1[#+]	M	73	Severe	15	21	44	N	171	13339	10211	281	8144	5422	575	48913	8208	1152
2[#+]	M	60	Severe	6	10	34	N	2590	8192	85	4309	7899	85	225	26461	85	1547
				12	16			6874	49384	85	38115	87297	85	1132	121091	106	n.d.
3*	F	60	Severe	8	15	11	Y	132	6985	639	227	11173	527	146	16734	413	163
4[#+]	M	48	Severe	6	13	48	N	169	4574	2495	1111	4728	4360	85	951	2417	663
				47	54			85	8295	696	85	3690	430	85	654	352	n.d.
5[#+]	F	56	Severe	8	20	16	N	1394	23884	3288	6009	23141	3844	545	2372	174309	1214
6*	M	52	Severe	5	12	20	N	85	85	194	372	21211	261	434	49733	1356	72
				8	15			147	1032	1112	325	21713	569	783	36023	6666	n.d.
7*	M	73	Severe	4	11	41	N	85	229	103	522	203	2268	85	21505	356	95
				7	14			232	5379	957	767	4308	5330	85	114266	715	n.d.
8*	F	47	Moderate	3	10	4	N	85	212	134	230	481	287	85	586	112	317
9	M	76	Severe	3	15	6	N	493	19108	2958	957	17623	2138	85	3688	7867	1378
10	F	46	Moderate	5	12	5	N	230	24830	9274	868	12756	6036	85	3647	5330	729
11	F	61	Severe	14	14	36	N	102	16815	438	238	12779	1354	85	7920	20096	401
				21	21			85	19784	516	85	8962	364	85	4447	7188	n.d.
12	M	46	Severe	13	20	5	N	85	22162	2642	252	337093	4357	287	120111	597	1341
13	M	69	Severe	19	18	20	N	415	13127	458	860	18168	858	85	157384	585	2205
14*	M	55	Severe	4	8	12	N	162	183	399	188	196	398	85	85	85	113
15*	F	54	Severe	25	28	40	N	151	13830	484	375	18608	3023	170	5689	9308	754
16*	M	44	Severe	2	3	3	N	134	163	85	80	1105	103	85	272	125	139
				27	28			194	10442	800	549	7479	291	85	5012	154	n.d.
17[#]	F	24	Moderate	3	6	2	N	1129	5437	2288	1712	737	1971	115	215	734	1020
18	M	62	Severe	23	57	35	N	85	7942	499	280	6954	464	85	16583	534	328
19	F	92	Severe	18	19	33	N	292	23860	1539	1361	19899	2935	85	25239	3451	1080
				28	29			221	45741	1448	325	26000	1379	104	17622	574	n.d.
20[#]	F	58	Severe	15	21	27	Y	255	58346	6803	1440	32416	3752	85	41945	2459	1026
21[#]	M	59	Severe	12	17	8	Y	1069	2612	7244	2174	871	5657	289	41431	8292	720
22	M	68	Severe	6	8	8	N	185	10906	274	308	10273	159	85	1989	368	905
23	M	78	Severe	5	43	24	N	85	5885	151	187	9767	280	85	12755	7586	281
24[#]	M	77	Severe	29	32	40	N	85	49325	645	85	48066	428	85	54024	4527	435
25	M	20	Severe	9	26	20	N	85	818	357	153	300	392	85	557	146	94
26	M	99	Severe	49	13	30	N	122	9887	5177	205	14989	1144	85	1283	365	283
27	F	85	Moderate	5	5	3	N	85	85	119	85	85	85	85	238	388	15
				18	18			245	3398	1674	235	2203	1184	85	142126	49883	n.d.
28	M	63	Severe	2	12	5	N	85	202	140	85	230	85	85	544	413	23
29	F	36	Severe	7	11	4	N	85	210	85	85	153	85	85	1342	85	82
30	M	41	Severe	5	7	5	N	104	85	136	85	407	85	85	324	85	163
31	M	26	Severe	3	11	5	N	85	617	675	85	349	135	85	321	85	102
32[#]	F	56	Severe	6	11	4	N	281	2366	1822	183	1145	1311	85	1562	140	111
33[#]	M	34	Severe	10	9	6	N	293	2736	2732	383	1302	728	85	770	202	403
				13	12			372	7292	1216	676	4305	1391	85	5137	320	n.d.
34[#]	M	62	Severe	4	10	33	Y	682	6126	1771	438	2642	218	154	1025	20215	503
				7	13			1598	23819	1248	849	6533	375	193	4764	18438	n.d.
				11	17			593	6229	934	646	5486	912	105	3230	49453	n.d.
35[#]	M	51	Severe	4	7	5	N	550	13780	695	813	14374	164	85	342	340	1230
36	M	48	Severe	4	11	6	N	85	682	1550	85	320	279	212	13527	2280	89
37	F	54	Severe	4	11	5	N	104	1761	402	85	719	114	85	405	151	297
38	F	49	Severe	4	11	9	N	917	3451	4321	709	883	1430	158	3256	4901	1134
39	F	73	Severe	4	18	6	N	85	2950	113	85	3729	85	85	109681	5668	205
40[#]	M	58	Severe	9	10	8	N	368	7193	863	256	9237	899	1537	7856	3483	432
41[#]	F	50	Severe	9	15	N/A	N/A	2129	63143	4718	707	28898	3074	85	41575	9134	1354
42	F	83	Severe	3	8	21	N	85	85	303	85	85	85	85	218	127	15
43	M	72	Severe	9	9	32	Y	85	85	85	85	85	85	85	227	85	15
44[#]	F	62	Severe	11	14	20	N	371	4691	18777	85	727	2588	85	2243	4434	954
45	F	65	Severe	9	19	12	N	85	85	85	85	85	144	85	85	85	28
46[#]	M	64	Severe	4	14	17	Y	184	1817	864	179	2197	85	1022	4851	3491	525
47[^#]	M	65	Severe	31	40	20	N	342	26380	1889	457	17252	1269	85	8305	383	596
48[^#]	M	55	Severe	31	46	2	N	955	9097	600	1216	4750	284	276	2928	1075	1007
49[^#]	F	36	nd	8	15	nd	nd	664	86698	313	nd	nd	nd	nd	nd	nd	2233
50[^#]	M	76	nd	7	17	nd	nd	1493	28352	6865	nd	nd	nd	nd	nd	nd	2561

Supplementary Table 1. Clinical & Serological Data Summary.

*Clinical data, RBD binding and neutralization previously published in Suthar, et al (2020)
^Individuals were discharged from hospital at time of sampling
#Individuals included in serum depletion analysis

CHAPTER 3

Pre-existing SARS-CoV-2 immunity influences potency, breadth, and durability of the humoral response to SARS-CoV-2 vaccination

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Summary

The ongoing SARS-CoV-2 pandemic highlights the importance of determining the breadth and durability of humoral immunity to SARS-CoV-2 mRNA vaccination. Herein, we characterize the humoral response in 27 naïve and 40 recovered vaccinees. SARS-CoV-2-specific antibody and MBC responses are durable up to six months, although antibody half-lives are shorter for naïve recipients. The magnitude of the humoral responses to vaccination strongly correlates with responses to initial SARS-CoV-2 infection. Neutralization titers are lower against SARS-CoV-2 variants in both recovered and naïve vaccinees, with titers more reduced in naïve recipients. While RBD is the main neutralizing target of circulating antibodies, Moderna-vaccinated naïves show a lesser reliance on RBD, with >25% neutralization remaining after depletion of RBD-binding antibodies. Overall, we observe that vaccination induces higher peak titers and improves durability in recovered as compared to naïve vaccinees. These findings have broad implications for current vaccine strategies deployed against the SARS-CoV-2 pandemic.

Keywords

SARS-CoV-2, mRNA vaccination, humoral response, RBD, memory B cells, antibody durability

Introduction

SARS-CoV-2 is an ongoing public health crisis with over 450 million infections and 6 million deaths attributed to the virus worldwide two years after its emergence (47). In numerous study cohorts, overwhelming evidence has illustrated the importance of antibodies targeting the trimeric spike (S) protein on the viral surface, especially the receptor binding domain (RBD), in controlling SARS-CoV-2 infections (285-288, 308). RBD-specific antibodies in circulation correlate strongly with viral neutralization across infection cohorts (156, 163), and monoclonal antibodies derived from RBD-specific memory B cells generated during infection have been consistently characterized as potent neutralizers with several either approved for use or currently in clinical trials for treatment of COVID-19 (156, 286-288). Given the clear importance of RBD-specific antibodies in the protective immune response to infection, eliciting a similar antibody repertoire through vaccination was hypothesized to provide comparable immunity. Several vaccines containing versions of the SARS-CoV-2 spike protein were approved at the end of 2020 as effective tools to manage viral spread and disease severity. Two widely available vaccines, Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b2, utilize an optimized mRNA platform to deliver their immunogen, a pre-fusion stabilized version of the SARS-CoV-2 S-protein (279, 280). While both vaccines initially reported similar efficacy in Phase III trials, comparisons of vaccine efficacy over time have reported Moderna as slightly more effective than Pfizer in preventing hospitalizations from COVID-19 (248, 279, 280). This difference in efficacy may be due to variability in dose, durability, and the dominant viral variant; however, differences in vaccine-induced immunity may also play a role.

The continuous evolution and emergence of SARS-CoV-2 variants has been an ongoing threat to the pre-existing immunity established within the population from both natural infection

and vaccination efforts. Two currently defined variants of concern (VOCs) are the recent omicron (B.1.1.529) variant, and the delta (B.1.617.2/AY) variant, which remains prevalent globally (276, 309). Initial studies investigating the ability of vaccine-induced responses to combat emerging variants have reported decreased potency against select variants with the most dramatic reductions seen against the beta (B.1.351) and omicron (B.1.1.529) variant (310-312,313). Understanding which components of vaccine-induced immunity are responsible for durable and cross-reactive responses is critical as countries continue to shift vaccination and treatment strategies including the recent approval of booster shots for several licensed vaccines.

In this study, we characterize the humoral response to vaccination in a cohort of SARS-CoV-2 recovered and naïve individuals receiving either Moderna's mRNA-1273 or Pfizer's BNT-162b2. We observed striking differences in both the early and long-term kinetics of the cellular and serological response to vaccination based on the absence or presence of pre-existing immunity to SARS-CoV-2. Interestingly, we find that the half-life of the antibody response is almost double in recovered as compared to naïve vaccinees between 1 and 6 months post-vaccination. Importantly, the magnitude of the humoral response to SARS-CoV-2 vaccination strongly correlated with the immune responses to initial SARS-CoV-2 infection. Depletion experiments illustrated that naïve vaccinees, particularly those receiving Moderna's mRNA-1273, tended to generate a substantial non-RBD neutralizing antibody fraction. These findings highlight potential differences in antibody repertoire breadth generated between recovered and naïve individuals vaccinated with the two mRNA vaccines and advances our understanding of potentially variable mechanisms of viral neutralization.

STAR Methods

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Author Jens Wrammert (jwramme@emory.edu).

Materials availability

No unique reagents were generated in this study.

Data and code availability

This paper does not report new data sets of a standardized datatype and does not report custom code. Any additional information required to reanalyze the data reported in this work paper is available from the Lead Contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study Population

A longitudinal cohort of recovered and naïve vaccinated individuals were recruited at Emory University with approval from the institutional review board (IRB00022371). Informed consent was obtained from all participants before conduct of study procedures. Further demographic details of this cohort can be found in Supplementary Table 1.

Cell Lines

VeroE6-TMPRSS2 cells were generated and cultured as previously described (314). Briefly, VeroE6-TMPRSS2 cells were generated by transfecting VERO E6 cells (ATCC CRL-1586) with pCAGGS plasmid in which chicken actin gene promoter drives the expression of an open

reading frame comprising Puromycin N-acetyl transferase, GSG linker, 2A self-cleaving peptide of thosea asigna virus (T2A), human transmembrane serine protease 2 (TMPRSS2). Two days post-transfection, cells were trypsinzed and transferred to a 100 mm dish containing complete DMEM medium (1x DMEM, Thermo Fisher, # 11965118, 10% FBS, 1x penicillin/streptomycin) supplemented with puromycin (Thermo Fisher, #A1113803) at a final concentration of 10 µg/ml. Approximately ten days later, individual colonies of cells were isolated using cloning cylinders (Sigma) and expanded in medium containing puromycin. Clonal cell lines were screened for expression of TMPRSS2 by flow cytometry. VeroE6-TMPRSS2 cells were cultured in complete DMEM in the presence of Gibco Puromycin 10mg/mL (# A11138-03). VeroE6-TMPRSS2 cells were used to propagate all virus stocks.

Viruses

nCoV/USA_WA1/2020 (WA/1), closely resembling the original Wuhan strain and resembles the spike used in the mRNA-1273 and Pfizer BioNTech vaccine, was propagated from an infectious SARS-CoV-2 clone as previously described (296). icSARS-CoV-2 was passaged once to generate a working stock. . The B.1.351 variant isolate, kindly provided by Dr. Andy Pekosz (John Hopkins University, Baltimore, MD), was propagated once to generate a working stock. hCoV-19/USA/PHC658/2021 (herein referred to as the B.1.617.2 variant) was derived from nasal swab collected in May 2021. Using VeroE6-TMPRSS cells, the B.1.617.2 variant was plaque purified directly from the nasal swab, propagated once in a 12-well plate, and expanded in a confluent T175 flask to generate a working stock. All viruses used in this study were deep sequenced and confirmed as previously described (314).

METHOD DETAILS

Sample preparation

Briefly, plasma and PBMC were isolated from peripheral blood collected in CPT or heparin tubes from these participants following infection or vaccination. CPT and heparin tubes were processed according to the manufacturer's protocol, and separated plasma and PBMCs were collected separately. PBMCs were treated with ACK lysis buffer (catalog no. 118-156-101, Quality Biological) for 5 min and washed three times with PBS with 2% FBS before counting and analysis by flow cytometry. PBMC and plasma were frozen at -80°C prior to long-term storage at -80°C (plasma) or in liquid nitrogen (PBMC).

Flow cytometry

Freshly isolated or thawed PBMCs were stained first for viability with LIVE/DEAD Fixable Yellow (Thermo Fisher Scientific) and then for markers with the following mAbs: IgA (IS11-8E10; Miltenyi Biotec), IgD (IA6-2; BD Biosciences), IgG (G18-145; BD Biosciences), IgM (MHM-88; BioLegend), CD3 (SK7, BD Biosciences), CD4 (RPA-T4, BD Biosciences), CD8 (SK1; BD Biosciences), CD14 (61D3; eBioscience), CD16 (CB16; eBioscience), CD19 (SJ25C1; BD Biosciences), CD20 (2H7; BD Biosciences), CD27 (O323; BioLegend or MT271; BD Biosciences), CD38 (HB7; BD Biosciences), and CD71 (CY1G4; BioLegend). Ag-specific B cells were detected by staining with RBD-conjugated to Alexa Fluor 488 (Alexa Fluor 488 Protein Labeling Kit; Thermo Fisher Scientific). RBD was conjugated as previously described.[308] After staining, PBMCs were washed and then fixed for 15 min using 2% paraformaldehyde (PFA; Thermo Fisher Scientific). Data were acquired on a BD FACSymphony A5 and analyzed using FlowJo 10.8.0 (BD Biosciences).

Antibody Binding Assay

Binding analyses were performed on plasma and serum samples using one or more of the following multiplexed antigen panels: V-PLEX COVID-19 Coronavirus Panel 1 (K15362/64U), V-PLEX SARS-CoV-2 Panel 7 (K15438U), V-PLEX SARS-CoV-2 Panel 11 (K15455U), and/or V-PLEX SARS-CoV-2 Panel 13 (K15463U). Briefly, plates were blocked with 150 µL/well of PBS + 5% BSA for 30 minutes shaking at 700 rpm. After washing 3x with PBS+0.05% Tween-20, 50 µL /well of sample diluted at 1:20,000 was added to the plate in duplicate and incubated for 2 hours shaking at 700 rpm. After washing, 50 µL /well of SULFO-TAG secondary (Anti-Human IgM, IgG, or IgA as appropriate) was incubated for 1 hour shaking at 700 rpm. After a final wash, 150 µL/well of MSD GOLD Read Buffer was added, and plates were read immediately on the MESO QuickPlex SQ 120. Antibody titers were calculated with Discovery Workbench 4.0 using a standard curve and are reported in arbitrary units per mL (AU/mL).

Focus Reduction Neutralization Test.

FRNT assays were performed as previously described (315). Briefly, samples were diluted at 3-fold in 8 serial dilutions using DMEM (VWR, #45000-304) in duplicates with an initial dilution of 1:10 in a total volume of 60 µl. Serially diluted samples were incubated with an equal volume of WA1/2020 or B.1.351 or B.1.617.2 (100-200 foci per well based on the target cell) at 37º C for 1 hour in a round-bottomed 96-well culture plate. The antibody-virus mixture was then added to VeroE6-TMPRSS2 cells and incubated at 37º C for 1 hour. Post-incubation, the antibody-virus mixture was removed and 100 µl of pre-warmed 0.85% methylcellulose (Sigma-Aldrich, #M0512-250G) overlay was added to each well. Plates were incubated at 37º C for 16 hours.

After 16 hours, methylcellulose overlay was removed, and cells were washed three times with PBS. Cells were then fixed with 2% paraformaldehyde in PBS for 30 minutes. Following fixation, plates were washed twice with PBS and 100 µl of permeabilization buffer, was added to the fixed cells for 20 minutes. Cells were incubated with an anti-SARS-CoV spike primary antibody directly conjugated with alexaflour-647 (CR3022-AF647) for up to 4 hours at room temperature. Cells were washed three times in PBS and foci were visualized and imaged on an ELISPOT reader (CTL).

RBD Depletion Assay

Depletion of RBD-specific antibodies from plasma was conducted as previously described (308). Briefly, plasma samples were diluted 1:10 with superparamagnetic beads coupled to RBD according to the manufacturer's protocol. Samples were incubated with rotation at RT for 1 hour after which the diluted plasma was separated from beads and transferred to tubes containing the same amount of RBD-coupled beads separated from storage buffer. Samples were incubated again rotating at RT for 1 hour, and the diluted plasma was separated from beads and transferred to fresh tubes for analysis. Removal of RBD-binding antibodies was confirmed through binding analysis (as described previously), and neutralization assays were performed as described previously using an initial dilution of 1:50 in 100 µL.

QUANTIFICATION AND STATISTICAL ANALYSIS

FRNT Quantification

Antibody neutralization was quantified by counting the number of foci for each sample using the Viridot program (316). The neutralization titers were calculated as follows: 1 - (ratio of the mean

number of foci in the presence of sera and foci at the highest dilution of respective sera sample). Each specimen was tested in duplicate. The FRNT-50 titers were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 9.2.0. Samples that do not neutralize at the limit of detection at 50% are plotted at the initial plasma dilution.

Statistics

Data were analyzed using GraphPad Prism 8.4.3. Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction for all comparisons of cell populations and antibody titers across timepoints. Brown-Forsythe ANOVA and Dunnet's T3 multiple comparison test or mixed-effects model with Geisser-Greenhouse correction was used to calculate statistics between groups. Pearson correlation coefficients and linear regressions were applied as appropriate. Mixed-effects models implemented in MonolixSuite 2020R1 (Lixoft) were used to estimate the corresponding half-lives of antigen-specific antibodies. The equation *A* dAb/dt=-k*Ab was fitted to the longitudinal data starting from day 21 after the second vaccine dose (data for each individual are shown in Figure 7 as circles connected with thin lines), where Ab is the antibody level and k is the exponential decay rate. The corresponding half-lives were calculated as t1/2=ln(2)/k. The individual-level parameters were lognormally distributed for the initial Ab level (at day 21) and normally distributed for the decay rate k with an assumption of no correlations between the random effects. We assumed multiplicative independent lognormal observation error. The estimation of the population parameters was performed using the Stochastic Approximation Expectation-Maximization (SAEM) algorithm, and corresponding fits are shown with thick lines in Figure 7.

Results

Study Cohort

We recruited at total of 67 individuals receiving a SARS-CoV-2 mRNA vaccine. Of these individuals, 39 were previously enrolled in our longitudinal study of COVID-19 immune durability study[135] and had previously been followed for up to 12 months after confirmed SARS-CoV-2 infection (median=296 days) post symptom onset. (Figure 1A). An additional 28 naïve participants were enrolled. These participants reported no known COVID-19 exposure. However, initial pre-screening identified one participant that exhibited serological and cellular evidence of previous infection. This participant was thus moved to the recovered group. These groups were vaccinated with either the Pfizer BNT162b2 (n=18 naïve, n=26 recovered) or Moderna mRNA-1273 (n=9 naïve, n=14 recovered). Gender-distribution was similar in both groups, with slightly more female participants (naïve=59%, recovered=53%) than male. The age of the naïve participants trended slightly younger, with a median age of 34 (range [22-64]) as compared to the recovered group median of 55 (range [21-77]). Further detailed information on all participants can be found in Supplemental Table 1. We collected plasma and peripheral blood mononuclear cell (PBMC) specimens from the participants at seven timepoints, including one pre-vaccination and six follow-ups (Figure 1A). Lymphocyte subsets were assessed by flow cytometry over the course of the study and remained stable (Supplemental Figure 1).

Moderna and Pfizer SARS-CoV-2 vaccines both induce durable RBD-specific memory B cell responses in SARS-CoV-2 naïve and recovered subjects.

Antigen-specific MBCs are known to be strongly induced by vaccination with significant increases from baseline reported (298, 317, 318). We assessed the magnitude and dynamics of RBD-specific MBCs in SARS-CoV-2 naïve and recovered subjects vaccinated with either the Moderna or Pfizer vaccine. Our gating strategy defined total MBCs as $CD19^{+}$, IgD^{-}, $CD20^{+}$ lymphocytes (Supplemental Figure 1). RBD-specificity was measured by staining with fluorescently labelled RBD (Figure 1C). Thirty-nine recovered individuals were recruited from a larger group of 55 participants followed longitudinally, as shown in Figure 1A, allowing us to link analyses of SARS-CoV-2 specific immune memory following infection with a subsequent vaccination. Subjects in both the recovered and naïve groups were assessed before and after each dose, then one, three, and six months following full vaccination (Figure 1). Recovered participants responded robustly to either vaccination, with RBD-specific MBC reaching a median of 0.42% of CD19+ cells in Pfizer-vaccinated subjects and 1.01% of CD19+ in Moderna-vaccinated subjects following the first dose. The second vaccine dose did not cause additional increases in RBD-specific MBCs for either vaccine. In contrast, RBD-specific MBC in naïve participants showed minimal responses after the first vaccine dose, instead peaking following dose 2, reaching medians of 0.14% (Pfizer) and 0.22% (Moderna) of CD19+ ($p<0.001$ Pfizer, $p=0.019$ Moderna, as compared to baseline) (Figure 1D). Despite minor differences in the median RBD-specific B cells across timepoints, there were no significant differences between Pfizer and Moderna in either the recovered or naïve groups. RBD-specific MBC responses were durable in both naïve and convalescent vaccinees, present in all groups three to six months after immunization (Figure 1D). To compare the durability of the RBD-specific MBC response in recovered and naïve individuals, we performed a linear regression analysis on timepoints collected between 1- and 6-months post-vaccination and compared the slopes of the generated

lines (Figure 1E). We observed a significant difference in slopes between recovered and naïve groups (p=0.0002) with RBD-specific MBCs slightly decreasing in recovered individuals over time and naïve individuals displaying an increase in RBD-specific MBCs up to 6 months post-vaccination, reaching comparable numbers as recovered individuals at this timepoint (Figure 1E).These data show that mRNA vaccination induces robust RBD-specific MBC formation in both recovered and naïve vaccinees, underlines that one dose may be sufficient for recovered individuals, and shows no significant difference between the two available mRNA-based vaccines.

The RBD-specific MBC response to vaccination is dominated by IgG in both naïve and recovered individuals.

To further characterize the RBD-specific MBC response, we separated the MBC compartment by expression of IgM, IgG, and IgA. We did not find significant RBD-specific IgM+ MBC, even at early timepoints. Though IgM+ MBC have been shown to form an important part of early immune responses (319, 320), responses to SARS-CoV-2 infection (135) and vaccination (321) are dominated by IgG+ MBC. RBD-specific IgG+ MBC formed the bulk of the response, driving the pattern of total RBD-specific MBC. Recovered individuals robustly responded to the first dose, increasing RBD-specific IgG+ MBC (p=0.002 Pfizer, p=0.008 Moderna), but did not increase further after dose 2 (Figure 1G). In naïve individuals, RBD-specific IgG+ MBC peaked following dose 2 (p<0.001 Pfizer, p=0.017 Moderna) and remained elevated at six months after vaccination (p<0.001 Pfizer, p=0.004 Moderna, as compared to baseline). IgA+ RBD-specific responses were more variable. Pfizer- and Moderna-vaccine responses did not differ significantly

from each other in either the naïve or recovered groups at any timepoint. Although not observed in Moderna recipients, the recovered Pfizer group exhibited a significant increase in IgA+ RBD-specific MBC over baseline after the first dose ($p=0.047$) and retained a small but significant increase six months after vaccination ($p=0.005$). Naïve vaccinees also generated slight but significant IgA+ RBD-specific MBCs six months following vaccination in both Moderna ($p=0.044$) and Pfizer (0.015) cohorts (Figure 1G).

RBD-specific MBC exhibit sustained activation, as measured by expression of CD71.

We have recently reported that RBD-specific MBC upregulate the activation marker CD71 during acute COVID-19.[308] To determine the duration of activation, we assessed CD71 expression in RBD-binding and non-RBD-binding MBC following SARS-CoV-2 infection and vaccination (Figure 2). One to two months after infection, a significantly higher percentage of RBD-specific MBCs retained expression of CD71 as compared to their non-RBD-binding counterparts ($p<0.0001$). This difference subsided over time but remained significant until 6-7 months after infection ($p=0.026$) suggesting prolonged antigenic stimulation. After vaccination, RBD-specific MBC activation mirrored the pattern of expansion shown in Figure 1E, with RBD-specific MBC showing the most CD71 expression post-dose 1 in the recovered ($p<0.0001$) and post-dose 2 in the naïve ($p<0.0001$) vaccinees (Figure 2B). We did not observe significant differences in CD71 expression between Pfizer- and Moderna-vaccinated groups, in either naïve or recovered vaccinees. In the recovered group, the second dose did not result in further upregulation of CD71 ($p>0.999$, timepoint 3 vs 4), and by 1 month post vaccination RBD-specific MBC trended higher in CD71 expression as compared to non-RBD-specific MBC, but

no longer reached significance (p=0.057). In comparison, RBD-specific MBC of naïve vaccinees remained significantly activated even six months after vaccination (p<0.0001).

Plasmablast expansion in peripheral blood is minor following SARS-CoV-2 vaccination.

An early measure of the humoral immune response is expansion of peripheral antibody secreting plasmablasts. Plasmablasts are typically observed early following vaccination (298, 322) and have been reported to expand in naïve vaccinees following the second dose of the Pfizer vaccine (323). In Figure 3B, we show plasmablasts as percentage of CD19+ for each naïve (top) and recovered (bottom) subject. Because peripheral plasmablasts are known to increase transiently, we excluded subject visits at timepoint 2 and 4 that were more than 14 days after the respective dose (Figure 3C). Plasmablasts responses observed in all groups were highly variable. Naïve vaccinees who received the Pfizer vaccine exhibited significantly expanded plasmablasts at timepoint 2 (p=0.001) and 4 (p=0.007). Overall, Moderna did not elicit a significant increase in plasmablasts in naïve subjects at any timepoint, but this difference may be due to the high variability of responses, with expansion in Moderna vaccinees ranging from 0.43% to 8.65% of B cells at timepoint 2 and ranging 0.55% to 4.42% at timepoint 4, combined with the comparatively lower number of subjects. Recovered subjects did not show significant plasmablast expansion at any timepoint (Figure 3C).

RBD-specific plasmablasts are detectable in the periphery of naïve and recovered subjects following vaccination.

Expansion of antigen-specific plasmablasts have been documented after infection (188) and vaccination (298, 322) for multiple pathogens and vaccines. We assessed RBD-specific plasmablasts following vaccination of SARS-CoV-2 naïve and recovered individuals, using optimized staining protocols to allow detection of RBD-specific plasmablasts in spite of low surface receptor expression. Despite a surprisingly low overall expansion of total plasmablasts, we found that a portion of the plasmablasts present following vaccination did exhibit binding to RBD. In particular, RBD-specific IgG-expressing plasmablasts were significantly expanded in recovered participants following the first dose of either mRNA vaccine ($p=0.014$ Pfizer, $p=0.036$ Moderna). In naïve individuals vaccinated with Pfizer, RBD-specific IgG+ plasmablasts significantly expanded following the second dose ($p<0.001$). Though some individuals vaccinated with Moderna did have RBD-specific plasmablast responses, overall there was no significant response following the second dose (Figure 3F). While the peripheral plasmablast response was generally minor, recovered subject responses occurred mainly after the first dose and naïve subjects after the second. In addition, the systemic source of the antibody response to vaccination may not be derived from circulating plasmablasts, but rather dependent on local germinal center and plasma cell formation as suggested by a recent report on vaccine responses in draining lymph nodes (323).

Vaccination induces robust IgG and IgA titers against SARS-CoV-2 S antigens in naïve and recovered individuals.

We conducted serological analyses using multiplexed antigen panels, containing SARS-CoV-2 S antigens (Spike N-Terminal Domain (NTD), RBD and S) in addition to SARS-CoV-2

nucleoprotein (N) and spike proteins derived from additional endemic and pathogenic coronaviruses. The kinetics of the antibody response resembled those observed in the RBD-specific MBC response, with antibodies targeting SARS-CoV-2 S antigens peaking in recovered individuals after a single vaccine dose. In contrast, naïve individuals required two doses to reach similar antibody levels as the recovered group (Figure 4A). Interestingly, we observed a similar pattern of increase in both antibodies against SARS-CoV-1 and, to a lesser extent, against MERS-CoV in both recovered and naïve individuals after vaccination (Supplemental Figure 2A). No significant changes were observed in antibodies against spike derived from either HKU1 or OC43, suggesting that vaccination has minimal effect on these pre-existing antibody titers (Supplemental Figure 2A). As expected, titers against N were unaffected throughout vaccination; recovered individuals displayed a higher baseline titer due to their previous exposure to SARS-CoV-2 (Supplemental Figure 2B).

IgM titers against RBD and S were significantly lower than IgG and IgA titers, rapidly declined, and returned to baseline by 1-month post-vaccination in both recovered and naïve groups (Supplemental Figure 2C). Anti-NTD, -RBD and -S IgG titers increased rapidly in recovered subjects following the first dose, increasing significantly compared to naïve subjects (Figure 4A). Following dose 2, IgG titers in naïve individuals were comparable to their recovered counterparts (Supplemental Table 7). IgG titers against NTD, RBD, and S fell more rapidly in naïve versus recovered groups, shown by significantly higher titers in recovered subjects one, three, and six months after vaccination (Figure 4A, Supplemental Table 7). IgA titers followed a similar pattern: recovered groups peaked following one dose while naïve groups required two. IgA titers did not significantly differ between recovered and naïve individuals post-dose 2 and continued to be comparable in both groups until six months post-vaccination (Figure

4A, Supplemental Table 7). IgG and IgA titers across almost all groups remained significantly higher baseline out to six months post-vaccination (Figure 4A). No difference in antibody titers was observed between the two different vaccines except in recovered individuals after the first dose (Supplemental Tables 4-6). At this point, S-specific IgG titers peaked in the recovered Moderna group as compared to the recovered Pfizer group, which continued to increase, reaching comparable titers prior to the second dose (Figure 4A, Supplemental Table 4). These data illustrate that, despite differing response kinetics, mRNA vaccination generated robust NTD-, RBD-, and S-specific antibody titers in both recovered and naïve individuals.

Neutralizing antibody titers against Beta and Delta variants are reduced in both recovered and naïve individuals

In addition to exploring antibody binding activity, we performed in vitro neutralization using a live virus assay on a subset of samples from our cohort taken at either 1 month post-infection (n=39) or post-vaccination (n=66). Samples were run against SARS-CoV-2 (WA1\2020). All vaccinated individuals had detectable neutralizing titers against SARS-CoV-2 at 1-month post-vaccination (Figure 4C). Recovered individuals had significantly higher titers than naïve individuals with no difference in titers between vaccine brand (Figure 4C, Supplemental Figure 3B). Neutralizing titers from both recovered and naïve individuals were significantly higher than titers from samples collected 1-2 months after initial infection with SARS-CoV-2 (Figure 4C). Additionally, we were able to compare neutralization titers between recovered (n=37) and naïve (n=25) vaccinees at 6 months after vaccination (Figure 4C). This data has recently been published in a study of Omicron neutralization in infected and vaccinated individuals and is

shown here for comparative reasons only (313). We observed that recovered individuals continued to have significantly higher neutralization that naïve individuals at this timepoint but note that the majority of individuals in both groups retain neutralizing titers against wild-type virus even 6 months from initial vaccination (Figure 4C).

We also assessed the binding and neutralizing response to SARS-CoV-2 variants, specifically beta (B.1.351) and delta (B.1.617.2/AY). We observed no difference in RBD binding to either variant over time in either naïve or recovered individuals (Supplemental Figure 3A). We conducted additional live virus neutralizing assays on a subset of our cohort (n=35) at 1-month post-vaccination using beta and delta variants of SARS-CoV-2. While we only observed a minimal fold decrease in variant binding at this timepoint, significant decreases in neutralizing titers were observed between WT and both beta and delta variants in recovered and naïve individuals regardless of the mRNA vaccine (Figure 4B, C). Titers were much lower in naïve individuals with an average fold reduction of 18.2 (beta) and 7.8 (delta) in the naïve group as compared to an average fold reduction of 7.8 (beta) and 5.8 (delta) in the recovered group (Figure 4B). Despite decreases in neutralizing capacity against the variants, all individuals retained neutralizing activity above the limit of detection against the delta variant, and all but one naïve individual retained activity against the beta variant (Figure 4C). Taken together, these data illustrate that neutralizing titers against variants are reduced but not ablated in both recovered and naïve individuals with recovered individuals possessing more robust variant-neutralizing fractions than their naïve counterparts.

Pre-existing humoral immunity correlates with B cell responses to SARS-CoV-2 vaccination.

To determine the correlation between humoral responses to infection and vaccination in our recovered individuals, we compared the relationship between RBD- and S-specific IgG titers at 1 month post-infection and 1 month-post vaccination. In agreement with our prior data (163, 308) and that of others (156), RBD and S-specific IgG titers correlated (r2=0.35, 0.49) with neutralizing titers post-infection (Figure 5A). A similar correlation was observed between RBD- and S-specific IgG and neutralizing titers post-vaccination in both recovered (r^2=0.55, 0.58) and naïve (r^2=0.16, 0.19) individuals (Figure 5A). We also compared the relationship between RBD-specific MBCs and RBD-specific IgG titers post-infection and vaccination (Figure 5B). In recovered groups, we observed correlations between levels of RBD-specific MBCs and IgG titers only post-vaccination (r^2=0.22) (Figure 5B). RBD^+ MBCs and IgG titers did not correlate in naïve vaccinees (p=0.73) (Figure 5B).

We also sought to determine if levels of binding titers, neutralizing titers, and antigen-specific MBCs prior to vaccination were predictive of the magnitude of response observed after vaccination in recovered individuals. We correlated these metrics between samples taken 1-month post-vaccination and either (a) samples collected 1-month post-infection or (b) samples collected within 1 month prior to vaccination (Figure 5C). We observed positive correlations across all metrics (RBD- and S-IgG, neutralization, and RBD^+ MBCs) between the post-infection and post-vaccination samples (Figure 5C). Similar positive correlations were observed between pre-vaccination and post-vaccination binding and neutralizing titers (Figure 5C). We also saw correlation between levels of RBD-binding MBC following infection and following vaccination (Figure 5D) No correlation was observed between pre-vaccination and post-vaccination levels of RBD^+ MBCs (Figure 5C), or RBD-specific MBCs pre-vaccination and RBD-specific IgG post-vaccination (Figure 5D). We additionally found no correlations between NTD-, RBD-, or S-

binding titers or RBD-specific MBCs and either age, gender, or time after infection (data not shown). These data provide evidence that the strength of the initial humoral response to SARS-CoV-2 infection predicts the strength of the response to vaccination in recovered individuals.

Vaccinated individuals display a range of dependency on RBD-binding antibodies for SARS-CoV-2 neutralization

In a previous study, we determined that the majority of the circulating neutralizing activity in acutely infected COVID-19 patients was driven by RBD-specific antibodies (308). Here, we sought to determine whether the circulating response to vaccination had a similar reliance on RBD-binding antibodies. We depleted RBD-specific antibodies, as previously described (308), from plasma in a subset of recovered (n=23) and naïve (n=12) individuals at 1-month post-vaccination and assessed subsequent binding and neutralization activity. We determined the efficacy of the depletion through a comparison of RBD-binding titers pre- and post-depletion (Figure 6A,B). RBD-specific IgM, IgG, and IgA titers were reduced significantly in all participants with average fold-changes of 15, 560, and 140-fold, respectively (Figure 6A, Supplemental Figure 4A). The depletion thus resulted in greater than a 97% reduction in RBD-binding IgG, with the majority of individuals exhibiting a 100% reduction in RBD-binding IgG post-depletion (Figure 6A). We then assayed neutralizing activity in pre- and post-depletion samples against SARS-CoV-2 (WA1\2020). Fold-change and percent reduction were calculated using pre- and post-depletion titers after subtracting the LOD. Neutralizing activity was reduced in all post-depletion samples regardless of previous SARS-CoV-2 exposure or vaccine brand (Figure 6B). Prior to factoring in vaccine brand, there was no significant difference in the percent

reduction of neutralization between recovered and naïve individuals. However, 82% (10/12) of naïve individuals retained >20% neutralizing capacity as compared to 52% (12/23) of recovered individuals (Figure 6C, D). When recovered and naïve individuals were split into subgroups based on vaccine received, we observed that the naïve Moderna group retained greater neutralization activity post-depletion than naïve (p=0.018) Pfizer vaccinees, with 66% (4/6) of vaccinees receiving Moderna retaining greater than 40% of neutralizing capacity post-RBD depletion (Figure 6C, 6D). As expected, this difference in RBD-dependency did not result in a significant correlation between binding or neutralizing titers against WT, beta, or delta variants and percent reduction of neutralization (Figure 6E, Supplemental Figure 4B). In addition, we also assayed binding titers towards SARS-CoV-2 S1 NTD, another epitope on the spike protein shown to elicit neutralizing antibodies (175, 324), and we again observed no significant correlation between IgG titers and percent reduction of neutralization (Figure 6E). Further insight into this difference between Moderna and Pfizer vaccine responses in naïve vaccinees requires additional work to characterize repertoire through single cell analyses. Taken together, this evidence illustrates that pre-existing immunity and vaccine brand both have an effect on the circulating repertoire of neutralizing antibodies produced in response to vaccination.

Pre-existing immunity impacts the durability of SARS-CoV-2 antibody and RBD-specific MBC levels.

A key factor in the continuing efficacy of any vaccine is the durability of the immune response it induces. Comparing the vaccination response of naïve to recovered individuals may also predict the response to a vaccine booster. We observed that levels of RBD-specific MBCs increase over

time in naïve individuals and parallels a sustained expression of CD71 on these MBCs (Figure 1G, 2B). This pattern contrasts with recovered vaccinees, whose RBD-specific MBCs decrease over time with CD71 expression quickly returning to baseline (Figure 1G, 2B). To compare durability of the antibody response between the recovered and naïve individuals, we fitted the data to an exponential decay model to determine the half-life of SARS-CoV-2 specific antibodies (Spike, RBD, NTD) (Figure 7). We found that recovered individuals had more durable antibody responses after vaccination than naïve individuals, with the half-life of antibodies in recovered individuals ($t_{1/2}$=95, 89, 88) almost double that of their naïve counterparts ($t_{1/2}$=47, 45, 52) (Figure 7). Overall, these observations illustrate that pre-existing immunity affects the long-term durability of the vaccine-induced immune response.

Discussion

The strength, breadth, and durability of the immune response to SARS-CoV-2 following vaccination is a topic of great importance as variants continue to emerge, and regulatory and governmental agencies across the world debate the benefits of booster shots for the general public. Additionally, the comparison of infection- versus vaccine-generated immunity is of public interest. Our study is uniquely suited to assess these factors due to the inclusion of a convalescent cohort followed for up to a year before vaccination as well as a naïve group. We have also included both Pfizer and Moderna vaccinees, to allow for a direct comparison of the induced immune response. To address the question of humoral and memory B cell durability, we tracked immune responses in our cohort up to six months after the completion of the mRNA vaccine regimen. Both recovered and naïve individuals experienced slight declines in RBD- and S-specific IgG and IgA titers from their peak response, with naïve individuals declining faster regardless of vaccine brand. Importantly, all individuals retained RBD- and S-specific titers at 6 months after vaccination that were significantly higher than baseline, suggesting that individuals would still have circulating antibodies that are likely protective from SARS-CoV-2 infection or severe COVID-19. We also assessed viral neutralization in both naïve and recovered groups. Though recovered individuals did exhibit higher levels of neutralization at 1-month post vaccination compared to naïve individuals, it is important to note that the naïve response to vaccination was significantly more neutralizing than that of the recovered group post-infection. This observation highlights that vaccine-generated immunity is as effective or better than the immunity following infection and raises the possibility that a booster in naïve individuals may induce a similar increase in neutralizing antibody. In addition, the majority of individuals in both

the recovered and naïve group retained neutralizing titers up to six months post-vaccination, further highlighting the durability of the vaccine-induced immune response.

Both naïve and convalescent individuals generated significant RBD-specific MBC, in levels comparable to SARS-CoV-2 infection (135) and influenza vaccination (298, 317, 318) In addition, RBD-specific MBCs in naïve individuals continue to increase after vaccination and are significantly elevated six months after immunization, reaching levels similar to that of the convalescent group. This increase in RBD-specific MBCs was also observed in individuals after a primary infection with SARS-CoV-2 (135, 325) Additionally, we found that a significant portion of those RBD-specific MBCs remain activated (CD71+) out to six months. These similar trends in antigen-specific MBC durability and activation are indicative of a sustained immune response months after initial exposure in both the case of primary infection and vaccination. Although further studies are needed to determine if this response is unique to SARS-CoV-2, our findings further highlight that vaccination alone produces memory B cell and antibody responses that are as good or better than infection alone at a magnitude comparable to other human infections (317, 318,298). It is likely that these cells will rapidly respond to either the administration of a third vaccine dose or a breakthrough SARS-CoV-2 infection, similarly to RBD-specific MBC expansion in recovered individuals following the first dose of a vaccine. The maintenance of a robust RBD-specific MBC population as circulating antibody titers begin to wane provides a potential explanation for the observation that, while vaccinated individuals can be infected with SARS-CoV-2, the disease severity is lower as compared to the unvaccinated (247).

The strength and kinetics of the immune response to initial SARS-CoV-2 vaccination remain important as public health organizations attempt to increase global vaccine access. We observed

stronger and more robust antibody responses in recovered individuals immunized with either mRNA vaccine as compared to those without a previous exposure, similar to recent reports (312, 326) and provide a more detailed kinetic analysis of the cellular and serological response during the course of vaccination. When comparing the humoral response between recovered and naïve individuals, we found that recovered individuals responded more strongly to the first dose of either mRNA vaccine than their naïve counterparts, with peak levels of both RBD-specific MBCs and RBD- and S-specific circulating IgG and IgA seen 1-3 wks after the first dose. In line with the induction of a potent immune response, we also observed peak levels of CD71 expression, a well-characterized marker of activation on B cells (299), on RBD-specific MBCs in recovered individuals after the first vaccine dose. These findings support the rapid recruitment and expansion of RBD-specific MBCs in response to vaccination in addition to increased RBD- and S-specific antibody production. Interestingly, we did not observe evidence of an additional immune response in recovered individuals after the second vaccine dose with no additional peak in either CD71 expression on MBCs or antibody titers. While the second vaccine dose could affect the durability of the vaccine-induced response, the results observed herein suggest that one dose of current mRNA vaccines is sufficient to boost SARS-CoV-2 immunity in previously infected individuals.

In contrast to their recovered counterparts, naïve individuals experienced peak levels in both RBD-specific MBCs and RBD- and S-specific antibody titers 1-2 wks after the second vaccine dose. This pattern is also evident in CD71 expression on RBD-specific MBCs, which increases progressively before peaking after the second dose. This observation illustrates that the RBD-specific MBCs generated are an active component of the immune response. Our finding that two mRNA vaccine doses are required for peak humoral immunity in naïve individuals is

well-supported by the requirement a two-dose regimen of both mRNA vaccines to reach robust levels of efficacy. While significant expansion of plasmablasts in peripheral blood has been reported in SARS-CoV-2 infection (308) and did occur in the naïve Pfizer vaccinees, mRNA vaccination did not appear to drive a robust RBD-specific plasmablast expansion in either naïve or recovered individuals. Though some individuals in both recovered and naïve groups had a high frequency of total plasmablasts occurring at one month following vaccination, these plasmablasts were not detectably RBD-binding and may be due to unrelated immune responses. Given that we observed high levels of RBD- and S-specific titers in circulation in both groups, it is unlikely that antibody-producing cells are absent, rather that vaccination is driving expansion in local sites such as lymph nodes near the site of vaccination, as has been observed in recent studies by Turner, et al. (323) Reasons for this lack of peripheral plasmablast expansion are unclear and warrants further examination.

In addition to the humoral durability to wild-type SARS-CoV-2, we sought to determine whether the immune response to vaccination remained effective against SARS-CoV-2 variants. The beta and delta variants of the virus have shown the most resistance to immunity generated from previous infection, with significant declines in neutralization (277, 327). A similar pattern of partial resistance has begun to be reported in studies of detailing the immune response to vaccination (277, 327, 328). While we observed no decrease in binding to variant RBD, we did find that both recovered and naïve individuals had significant decreases in neutralization towards both beta and delta variants at 1-month after vaccination. Additionally, naïve individuals experienced a steeper decline in variant-resistant neutralization with greater fold-change between wild-type and both variants observed in naïve vaccinees as compared to recovered. These observations suggest that recovered individuals may develop and retain an antibody population

that is more resistant to emerging SARS-CoV-2 variants than naïve individuals in response to vaccination, most likely due to the boosting of pre-existing antibody repertoire developed during infection.

To further assess differences in repertoire breadth between naïve and recovered individuals, we depleted RBD-specific antibody from the plasma of naïve and recovered vaccinees and determined neutralizing capacity. We previously published that neutralizing activity in acute SARS-CoV-2 patients is highly dependent on RBD-specific antibody (308). Similarly, the recovered group largely lost neutralizing activity when RBD-specific antibody was depleted, suggesting that vaccination in recovered individuals is likely recalling B cell responses established during previous infection. However, naïve individuals vaccinated with Moderna were able to retain more plasma neutralizing capacity in the absence of RBD-binding antibodies, suggesting that they may produce an antibody response with greater breadth. In the recovered group, responses to vaccination highly correlated to pre-existing immunity, both post-infection and immediately preceding vaccination. This correlation, the recovered group's dependence on RBD-specific antibody for neutralization, and our previous finding that acute SARS-CoV-2 patients also exhibit similar RBD-specific antibody dependence support that mRNA vaccination is skewing the antibody repertoire towards RBD-specificity through the recruitment of pre-existing clones as opposed to *de novo* activation of naïve B cells. Naïve Pfizer vaccinees also exhibited significantly more RBD-specific antibody-dependent neutralization, as compared to their Moderna-vaccinated counterparts. This difference may be due to the different formulations or dosages of the vaccines. The exact implication of these differences is as yet unclear given that they failed to correlate with binding or neutralizing titers to either wild-type or variant SARS-

CoV-2. Further experiments to determine the antibody specificities of these non-RBD neutralizers will be needed to ascertain their significance to the overall immune response.

A continuous debate throughout the development of SARS-CoV-2 vaccines is whether one vaccine brand elicits broader or more durable immune response than the other. In our study, we were able to compare the two mRNA vaccines available, Moderna's mRNA-1276 and Pfizer's BNT16b2, in both recovered and naïve individuals. We observed no difference in the generation of a robust RBD-specific MBC population in response to vaccination nor did we observe significant differences in ability of individuals to neutralize wild-type or variant SARS-CoV-2 1 month after vaccination, suggesting that both mRNA vaccines elicit a strong immune response against SARS-CoV-2. However, we did find that the S-specific IgG response in recovered individuals receiving Moderna peaked significantly faster than in individuals receiving Pfizer. This difference could likely be explained by the higher dosage (100 μg) of immunogen given in mRNA-1276, and the impact of this faster and stronger peak is unclear as S-specific titers between two vaccine groups normalize quickly. We also found that naïve Moderna vaccinees retained greater neutralizing titers in the absence of RBD-binding antibodies than both naïve and recovered Pfizer vaccinees. This finding points to a potential difference in the breadth of the antibody repertoire generated between Moderna and Pfizer. While we were unable to identify the epitope specificities of this non-RBD fraction, it is interesting to note this difference in the face of recent reports that Moderna is more efficacious in preventing hospitalization when compared to Pfizer, especially many months out from vaccination (248). Further investigation into the specific differences in repertoire generated by these two vaccines is necessary.

SARS-CoV-2 continues to be a critical worldwide public health threat. Vaccination, especially with the highly efficacious mRNA vaccines, remains the best possible strategy for

combatting the continuing pandemic. The comparison of antibody-binding, B cell memory, and neutralizing activity in recovered and naïve individuals provides a possible prediction of how individuals may respond to repeated exposure, either through vaccination or infection. Our study also underlines that vaccination is equal or better at inducing immunity as compared to infection alone, examines specificity of neutralizing activity in both Pfizer and Moderna immunization, and highlights the durability of the humoral immune response to vaccination.

Limitations of the study

Limitations of this study include a bias towards Pfizer vaccination in both naïve and recovered groups and a small sample size. In addition, while our study evaluates vaccine responses in both SARS-CoV-2 recovered and naïve groups, it reports only up to six months following vaccination and does not assess booster responses. Because this study continues to collect participant samples, including following booster administration, we will be able to further assess and evaluate our predictions on this important immune response.

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Author Contributions

G.M. and L.E.N contributed to the acquisition, analysis, and interpretation of data and writing the manuscript. V.V.E., K.E.M. and M.E. performed *in vitro* neutralization experiments. M.W.F. and C.N. contributed to data acquisition and editing the manuscript. V.I.Z. performed half-life calculations and approval of the final manuscript. C.R.C., L.H., and K.S. provided clinical support for the study and contributed to sample collection. E.J.A., R.A., M.S.S., and J.W. contributed to the conception and design of the work and the writing and approval of the final manuscript.

Declaration of Interests

E.J.A has consulted for Pfizer, Sanofi Pasteur, Janssen, and Medscape, and his institution receives funds to conduct clinical research unrelated to this manuscript from MedImmune, Regeneron, PaxVax, Pfizer, GSK, Merck, Sanofi-Pasteur, Janssen, and Micron. He also serves on a safety monitoring board for Kentucky BioProcessing, Inc. and Sanofi Pasteur. His institution has also received funding from NIH to conduct clinical trials of Moderna and Janssen COVID-19 vaccines. M.S.S serves on the advisory board for Moderna.

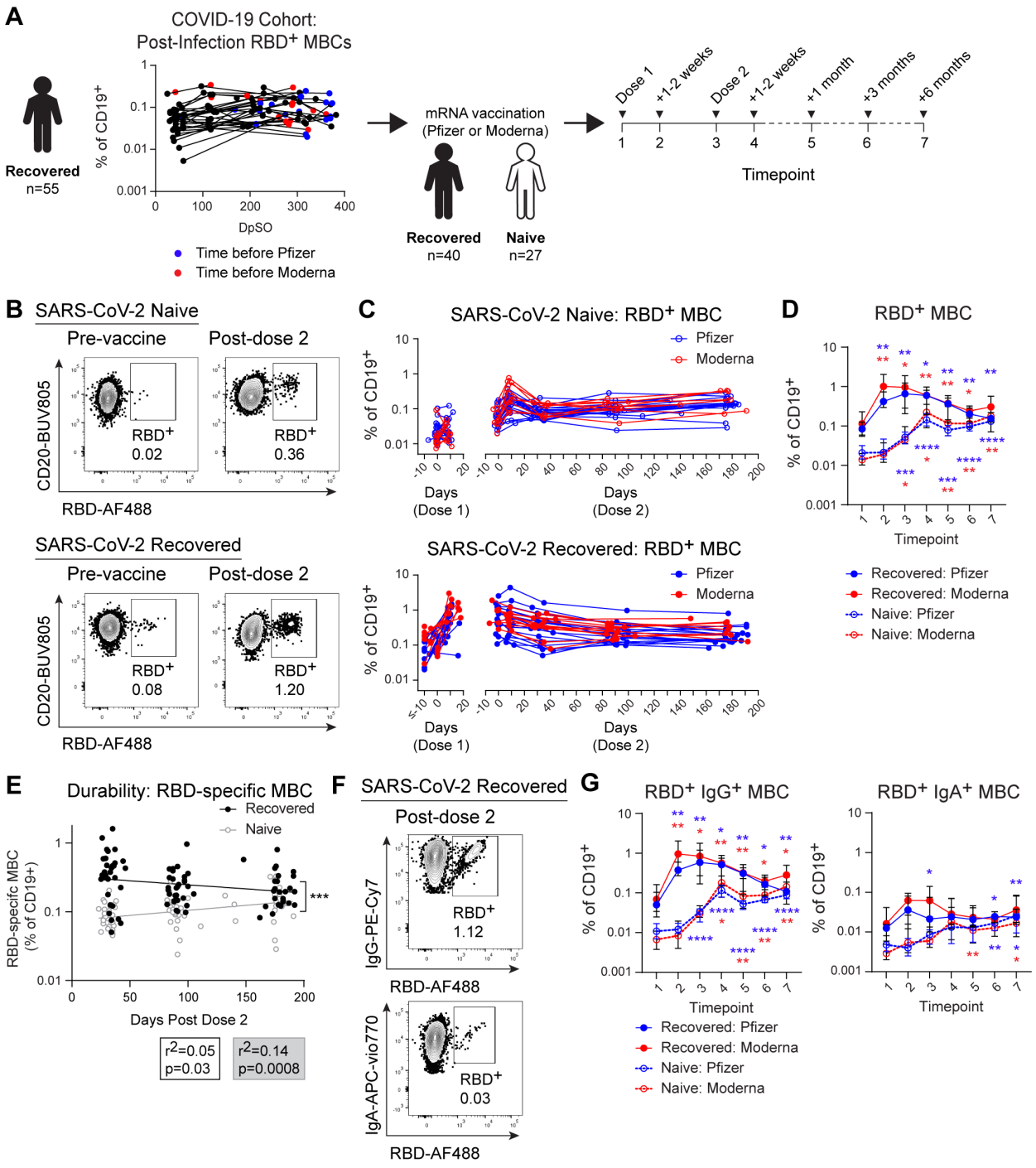

A
COVID-19 Cohort:
Post-Infection RBD+ MBCs
Recovered
n=55
% of CD19+
DpSO
Time before Pfizer
Time before Moderna
mRNA vaccination
(Pfizer or Moderna)
Recovered
n=40
Naive
n=27
Dose 1
+1-2 weeks
Dose 2
+1-2 weeks
+1 month
+3 months
+6 months
Timepoint
B
SARS-CoV-2 Naive
Pre-vaccine
Post-dose 2
CD20-BUV805
RBD-AF488
RBD+
0.02
RBD+
0.36
SARS-CoV-2 Recovered
RBD+
0.08
RBD+
1.20
C
SARS-CoV-2 Naive: RBD+ MBC
Pfizer
Moderna
Days
(Dose 1)
Days
(Dose 2)
SARS-CoV-2 Recovered: RBD+ MBC
D
RBD+ MBC
Timepoint
Recovered: Pfizer
Recovered: Moderna
Naive: Pfizer
Naive: Moderna
E
Durability: RBD-specific MBC
Recovered
Naive
RBD-specifc MBC
(% of CD19+)
Days Post Dose 2
r2=0.05
p=0.03
r2=0.14
p=0.0008
F
SARS-CoV-2 Recovered
Post-dose 2
IgG-PE-Cy7
RBD+
1.12
RBD-AF488
IgA-APC-vio770
RBD+
0.03
RBD-AF488
G
RBD+ IgG+ MBC
RBD+ IgA+ MBC
Timepoint
Recovered: Pfizer
Recovered: Moderna
Naive: Pfizer
Naive: Moderna

Figure 1. RBD-specific memory B cells expand in naïve and recovered subjects following mRNA vaccination. A) Study design of the Emory SARS-CoV-2 convalescence and vaccination cohort. Frequency of RBD-specific MBCs in subjects for 1-12 months following confirmed SARS-CoV-2 infection, reported as % of total CD19+ cells. The final visit before vaccination is shown in blue (Pfizer) or red (Moderna). B) Gating scheme of RBD-specific memory B cells, pre-gated as live single CD19+IgD-CD20+ cells, in a naïve (top) and recovered (bottom) donor before vaccination (left) and after dose 2 (right). C) RBD-specific MBCs as % of CD19 for individual vaccinated donors by days post dose 1 and 2. D) RBD-specific MBCs as % of CD19+ by timepoint. E) RBD-specific MBCs shown for naïve (grey, open circles) and recovered (black, closed circles) for 1-6 months following full vaccination. Coefficient of determination (r^2) and significance are determined from linear regression analysis. ***$p<0.001$ F) Gating scheme of RBD-specific IgG (left) or IgA (right) expressing MBC, shown for a recovered subject post-dose 2. G) RBD-specific IgG+ (left) or IgA+ (right) MBC as % of CD19+ by timepoint. All panels: Blue=Pfizer vaccination (recovered n=18, naïve n=18), red=Moderna (recovered n=13, naïve n=9), tested in singlets. Open circles are naïve subjects, closed circles are recovered. D,F) Values are medians ± 95% CI. Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction. Blue values indicate Pfizer, red values indicate Moderna, as comparisons to timepoint 1. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

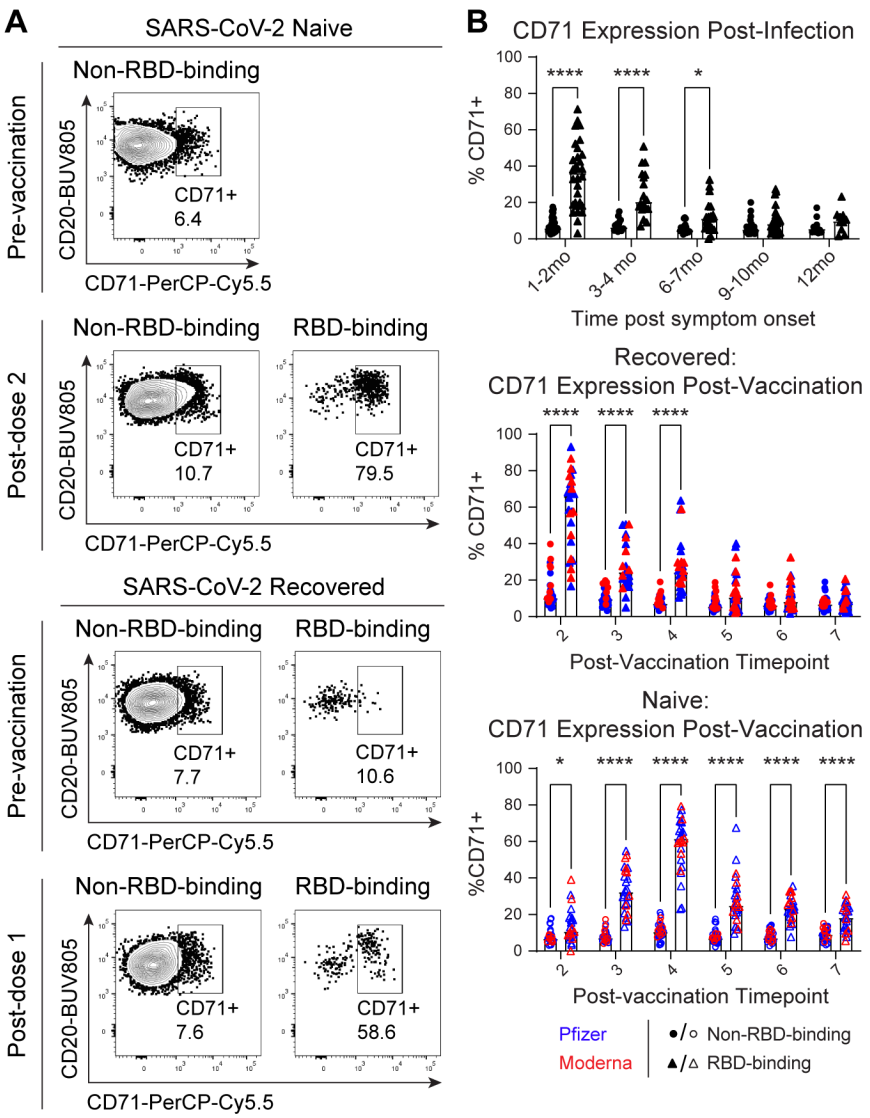


Figure 2. RBD-specific memory B cells upregulate CD71 following infection and vaccination. A) Gating scheme for CD71 expression on non-RBD binding (left) and RBD-binding (right) MBC. B) CD71 expression as percent of parent is quantified in RBD-binding (triangles) and non-RBD-binding (circles) following SARS-CoV-2 infection (top) or vaccination of recovered (middle) and naïve (bottom) subjects. Blue=Pfizer vaccination (recovered n=18, naïve n=18), red=Moderna (recovered n=13, naïve n=9), tested in singlets. Open symbols are naïve subjects , closed symbols are recovered. Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction. *p<0.05, ****p<0.0001

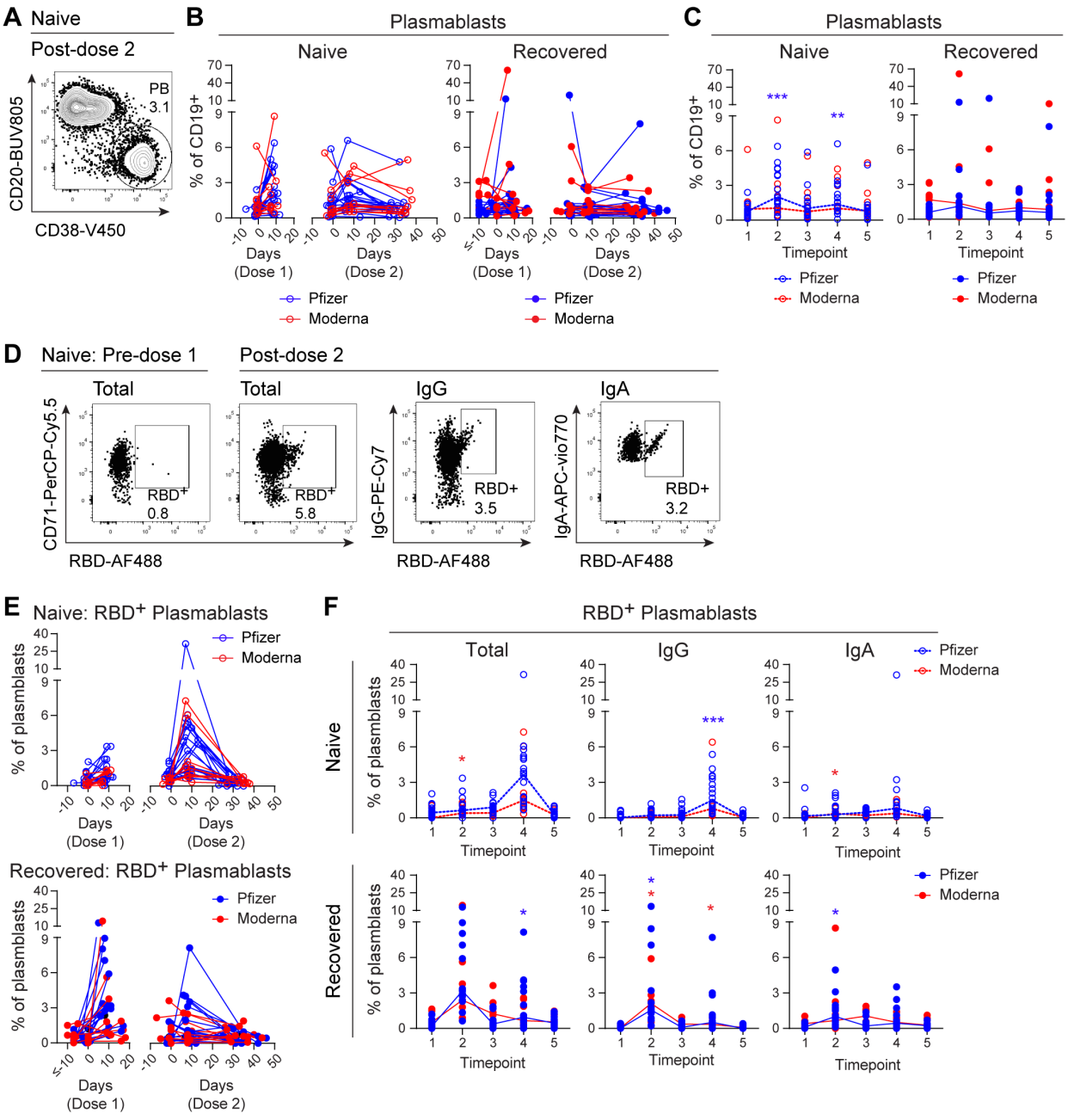

A Naive
Post-dose 2
PB
3.1
CD20-BUV805
CD38-V450
B
Plasmablasts
Naive
Recovered
% of CD19+
Days
(Dose 1)
Days
(Dose 2)
Pfizer
Moderna
C
Plasmablasts
Naive
Recovered
% of CD19+
Timepoint
Pfizer
Moderna
D
Naive: Pre-dose 1
Post-dose 2
Total
Total
IgG
IgA
CD71-PerCP-Cy5.5
IgG-PE-Cy7
IgA-APC-vio770
RBD-AF488
RBD+
0.8
RBD+
5.8
RBD+
3.5
RBD+
3.2
E
Naive: RBD+ Plasmablasts
Recovered: RBD+ Plasmablasts
% of plasmblasts
Days
(Dose 1)
Days
(Dose 2)
Pfizer
Moderna
F
RBD+ Plasmablasts
Total
IgG
IgA
Naive
Recovered
% of plasmblasts
Timepoint
Pfizer
Moderna

Figure 3. Plasmablast responses in naïve and recovered individuals post SARS-CoV-2 mRNA vaccination. A) Gating scheme of plasmablasts by CD20 and CD38, pre-gated as CD19+IgD-, in a representative naïve post-dose 2 (top) or recovered post-dose 1 (bottom). B) Plasmablasts as % of CD19 for individual vaccinated donors by days post dose 1 and 2. C) Plasmablasts as % of CD19+ by timepoint in naïve (left) and recovered (right) vaccinees. D) Gating scheme of RBD-specific plasmablasts pre- and post-vaccination in a naïve subject. E) RBD-specific total (left), IgG+ (middle), and IgA+ (right) as % of plasmablasts by timepoint in naïve (top) and recovered (bottom) vaccinees. All panels: Blue=Pfizer vaccination (recovered n=18, naïve n=18), red=Moderna (recovered n=13, naïve n=9), tested in singlets. Open circles are naïve subjects, closed circles are recovered. C, F) Statistics were calculated using mixed-effects model with Geisser-Greenhouse correction. Blue values indicate Pfizer, red values indicate Moderna, as comparisons to timepoint 1. *$p<0.05$, **$p<0.01$, ***$p<0.001$

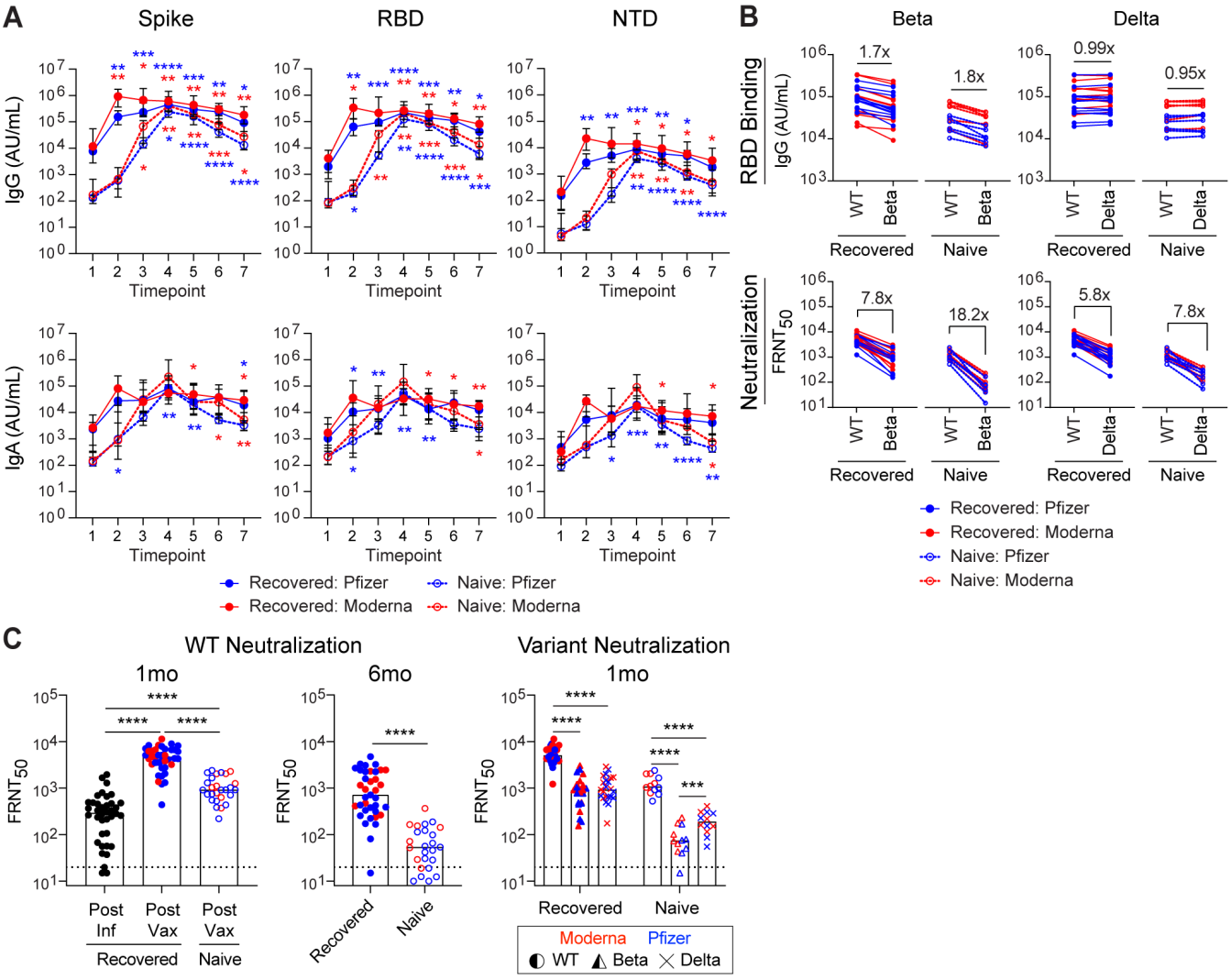

A
Spike
RBD
NTD
IgG (AU/mL)
IgA (AU/mL)
Timepoint
Recovered: Pfizer
Recovered: Moderna
Naive: Pfizer
Naive: Moderna
B
Beta
Delta
RBD Binding
IgG (AU/mL)
Neutralization
FRNT50
1.7x
1.8x
0.99x
0.95x
7.8x
18.2x
5.8x
7.8x
WT
Beta
Delta
Recovered
Naive
C
WT Neutralization
Variant Neutralization
1mo
6mo
FRNT50
Post Inf
Post Vax
Recovered
Naive
Moderna
Pfizer
WT
Beta
Delta

Figure 4. Recovered individuals generate faster and more robust antibody responses to mRNA vaccination against SARS-CoV-2 than naïve individuals. (A) IgG and IgA NTD-, RBD-, and S-specific binding titers over the course of vaccination in recovered (Pfizer=18, Moderna=10) and naïve (Pfizer=18, Moderna=9) individuals as determined by MSD-ELICA calculated from reference standard curve. (B) IgG binding and neutralizing titers against WT, b, and d RBD in recovered (Pfizer n=12, Moderna n=11) and naïve individuals (Pfizer n=6, Moderna n=6) at 1mo post-vaccination as determined by MSD-ELICA and *in vivo* neutralization (C) Neutralization titers in recovered (1mo post-infection (n=39), 1mo post-vaccination (n=39), 6-mo post vaccination (n=37)) and naïve (1mo post-vaccination (n=27), 6-mo post vaccination (n=25)) individuals against SARS-CoV-2 WT, beta, or delta variants. The 6-mo data for the WT virus was previously published in Edara, et al [313] and is shown here for comparative reasons only. MSD-ELICA and neutralization assays were run in duplicate. Significance was determined using either as differences from baseline (T1) using mixed-effects model with Geisser-Greenhouse correction and Tukey's multiple comparison test or using (i) Brown-Forsythe ANOVA and Dunnet's T3 multiple comparison test and (ii) two-way ANOVA with Geisser-Greenhouse correction. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

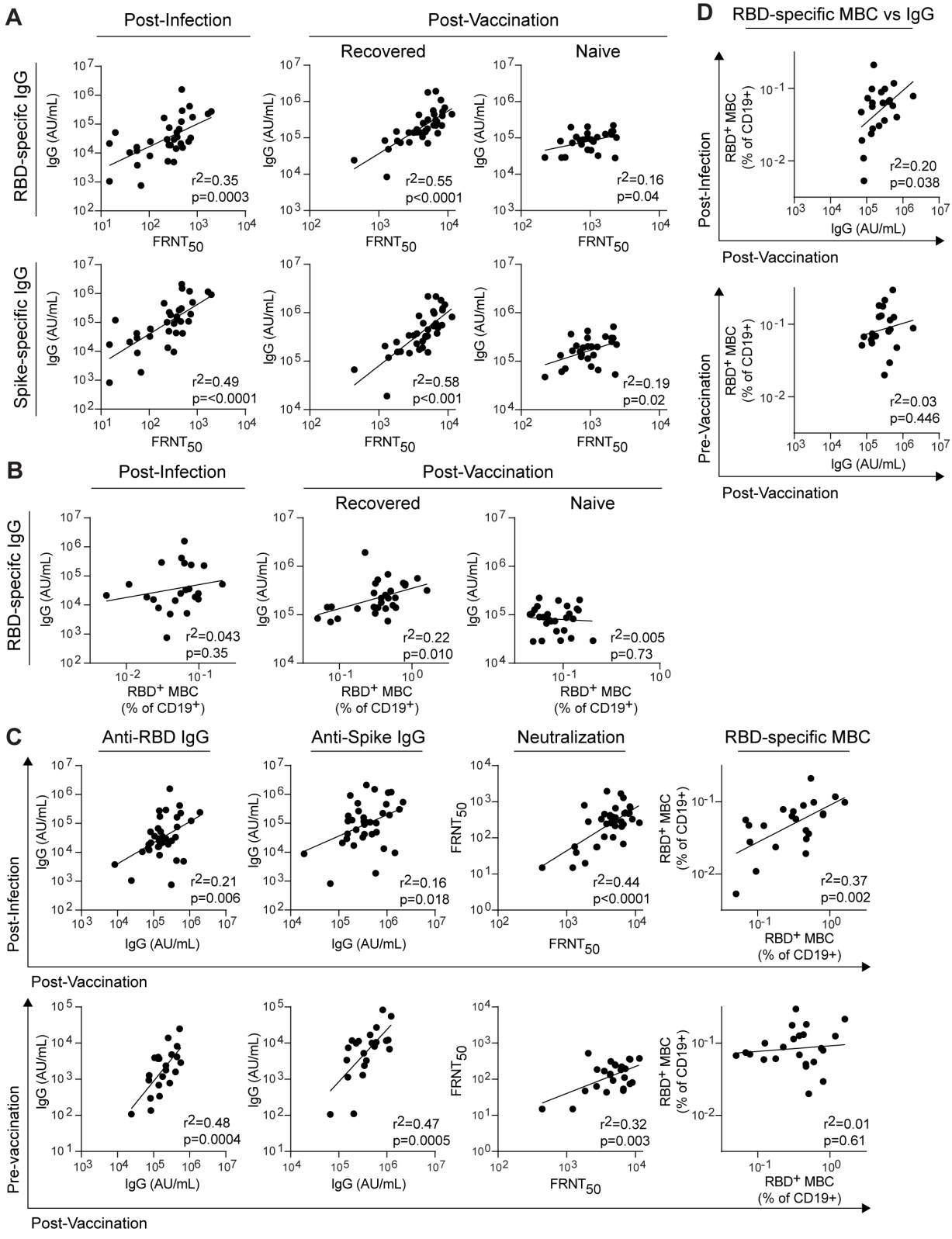

A
Post-Infection
Post-Vaccination
Recovered
Naive
RBD-specifc IgG
Spike-specific IgG
IgG (AU/mL)
FRNT50
r2=0.35
p=0.0003
r2=0.55
p<0.0001
r2=0.16
p=0.04
r2=0.49
p=<0.0001
r2=0.58
p<0.001
r2=0.19
p=0.02
D
RBD-specific MBC vs IgG
Post-Infection
Post-Vaccination
Pre-Vaccination
RBD+ MBC
(% of CD19+)
r2=0.20
p=0.038
r2=0.03
p=0.446
B
r2=0.043
p=0.35
r2=0.22
p=0.010
r2=0.005
p=0.73
C
Anti-RBD IgG
Anti-Spike IgG
Neutralization
RBD-specific MBC
r2=0.21
p=0.006
r2=0.16
p=0.018
r2=0.44
p<0.0001
r2=0.37
p=0.002
r2=0.48
p=0.0004
r2=0.47
p=0.0005
r2=0.32
p=0.003
r2=0.01
p=0.61

Figure 5. Antigen-specific MBC and serological responses to SARS-CoV-2 infection correlate with responses to mRNA vaccination. (A) RBD- and S-titers correlate with neutralizing titers 1mo post-infection (left) and 1mo post-vaccination in recovered (middle) and naïve (right), (B) RBD+ MBCs (% of CD19+) correlated with RBD-specific IgG titers (left) and 1mo post-vaccination in recovered (middle) and naïve (right) , (C) RBD- and S-binding titers, neutralizing titers, and RBD+ MBCs (% of CD19+) correlated between 1mo post-infection and 1mo post-vaccination (top) and 1mo pre-vaccination and 1mo post-vaccination (bottom). D) RBD-binding B cells post-infection (top) and pre-vaccination (bottom) are correlated with RBD-specific IgG following vaccination. Coefficient of determination (r^2) and significance determined from linear regression analysis.

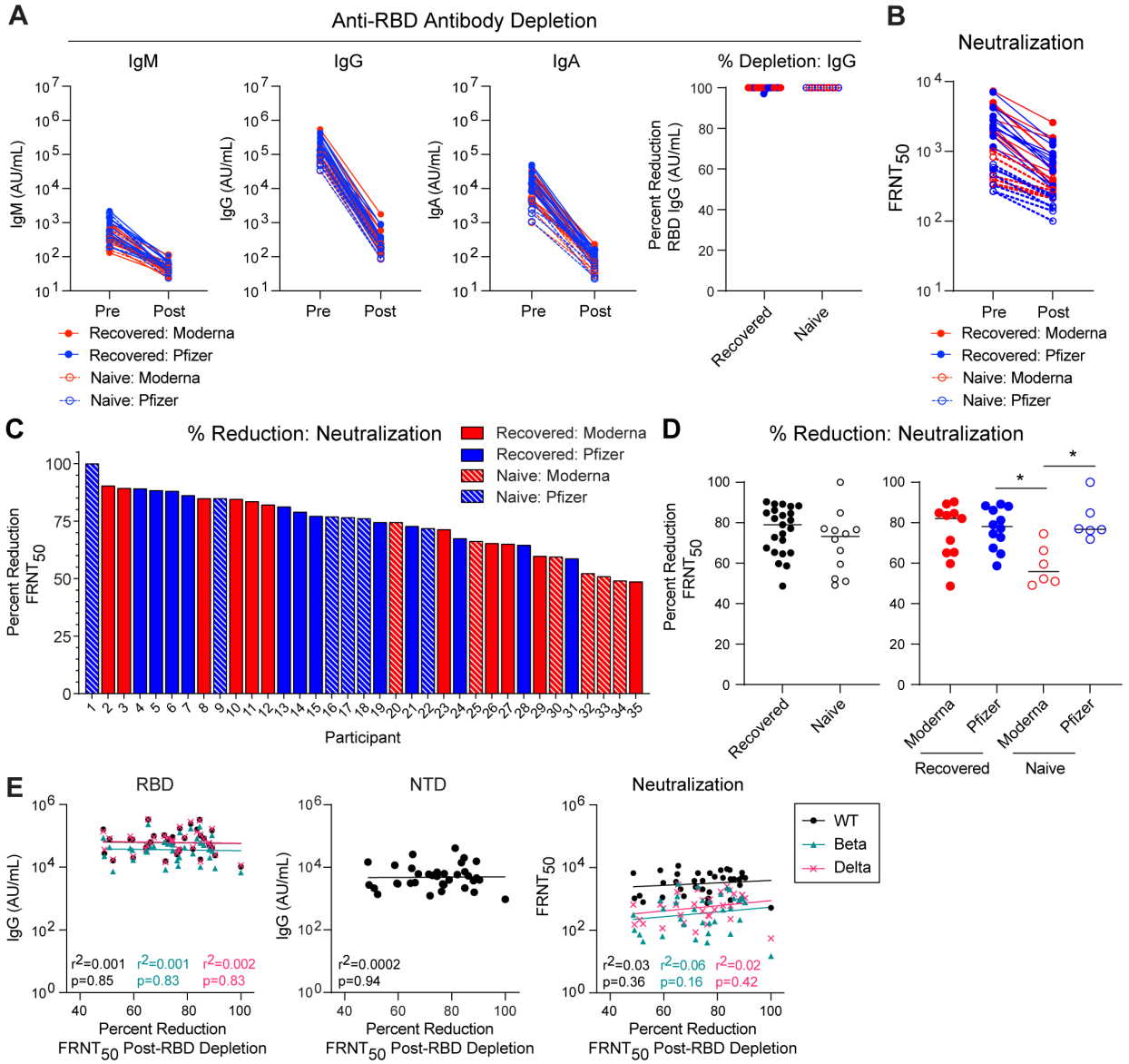

A
Anti-RBD Antibody Depletion
IgM
IgG
IgA
% Depletion: IgG
IgM (AU/mL)
IgG (AU/mL)
IgA (AU/mL)
Percent Reduction RBD IgG (AU/mL)
Pre
Post
Recovered
Naive
Recovered: Moderna
Recovered: Pfizer
Naive: Moderna
Naive: Pfizer
B
Neutralization
FRNT50
C
% Reduction: Neutralization
Percent Reduction FRNT50
Participant
D
% Reduction: Neutralization
Moderna
Pfizer
E
RBD
NTD
Neutralization
WT
Beta
Delta
r2=0.001 p=0.85
r2=0.001 p=0.83
r2=0.002 p=0.83
r2=0.0002 p=0.94
r2=0.03 p=0.36
r2=0.06 p=0.16
r2=0.02 p=0.42
Percent Reduction FRNT50 Post-RBD Depletion

Figure 6. Naïve Moderna-vaccinated individuals retain greater neutralizing capacity after depletion of RBD-specific fraction of plasma. (A) MSD-ELICA calculated titers for RBD-specific IgM, IgG, and IgA before (pre) and after (post) RBD depletion (left) and % reduction of RBD-specific IgG post RBD-depletion as calculated from fold change (right). (B) Neutralization titers against SARS-CoV-2 WT pre and post RBD depletion. (C, D) % reduction of neutralization post RBD-depletion as calculated from fold change. (E) IgG titers against RBD [WT, b, d] and neutralization titers [WT, b, d] correlated to % reduction of neutralization post RBD-depletion; coefficient of determination (r^2) and significance determined from linear regression analysis. Blue=Pfizer vaccination (recovered n=12, naïve n=6), red=Moderna (recovered n=11, naïve n=6), tested in duplicate. Open circles are naïve subjects, closed circles are recovered. Statistics were calculated by Brown-Forsythe ANOVA and Dunnet's T3 multiple comparisons (A-D). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

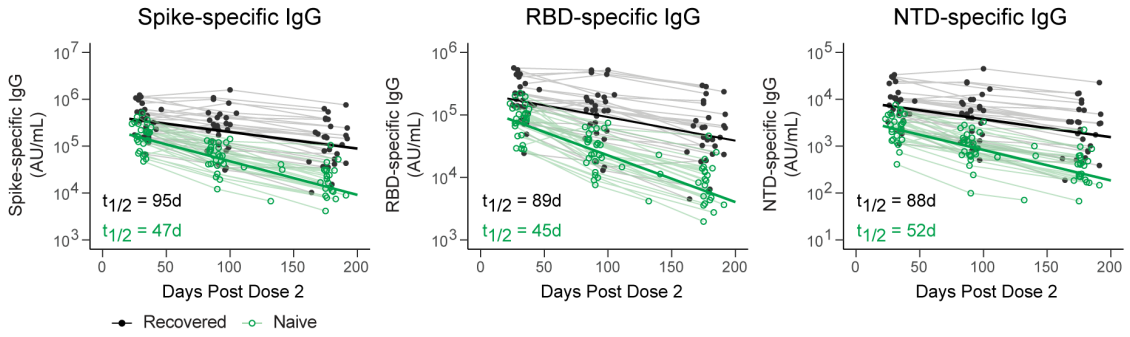
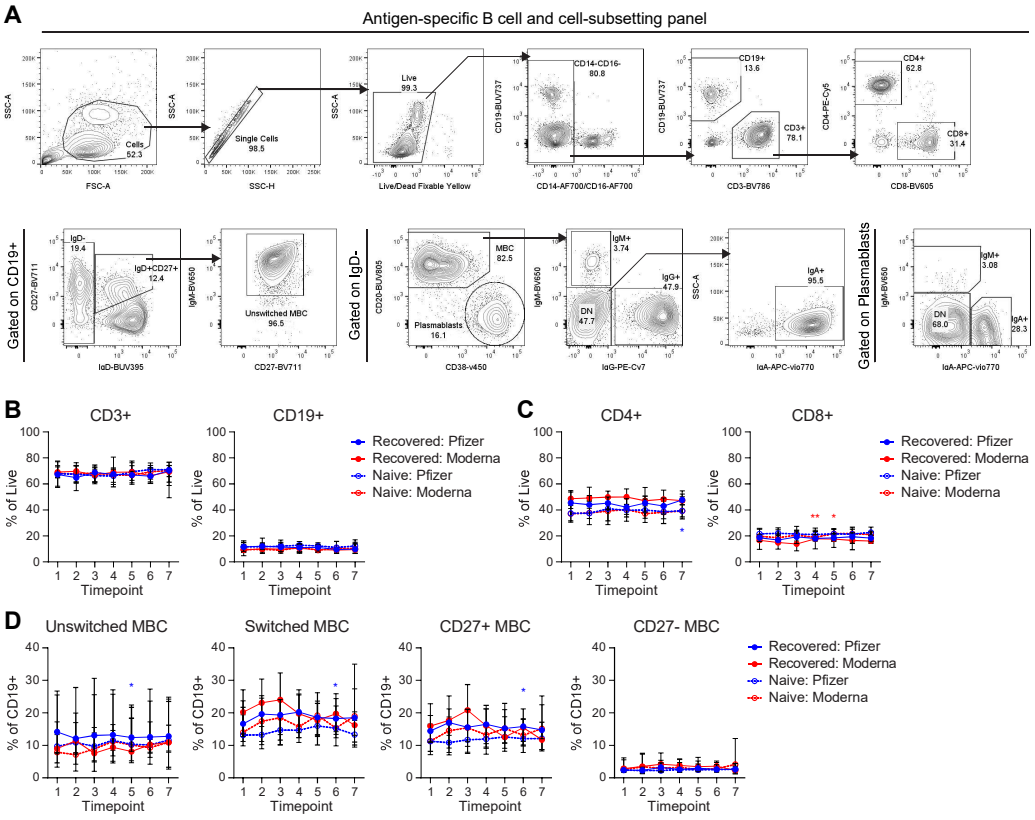
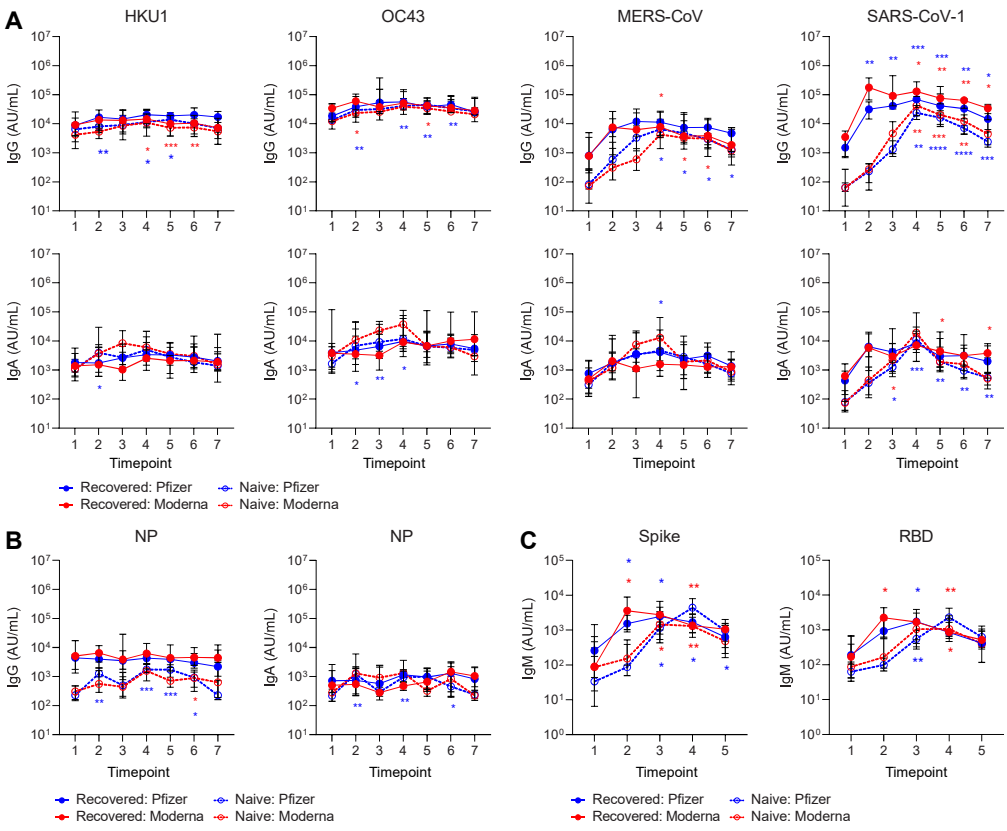


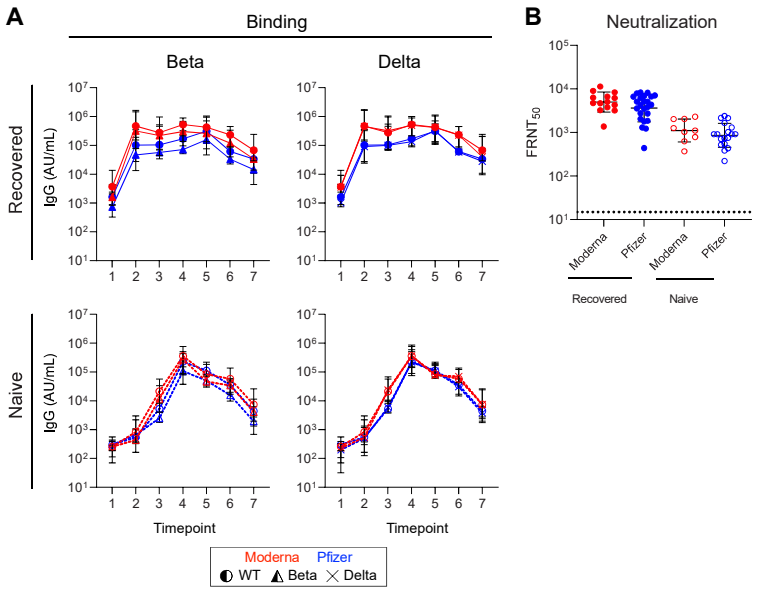
Figure 7. Naïve vaccinees exhibit a faster decline in Spike, RBD, and NTD-specific IgG six months after vaccination than previously SARS-CoV-2 infected vaccinees. Spike-specific IgG (left), RBD-specific IgG (middle), and NTD-specific IgG (right) is shown for naïve (green, open circles) and recovered (black, closed circles) for 1-6 months following full vaccination. Best-fit lines determined using an exponential decay model and antibody half-lives ($t_{1/2}$) calculated.



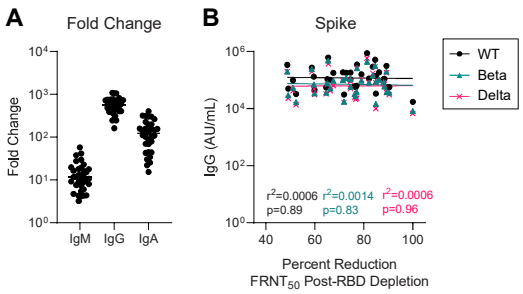
Supplemental Figure 1. Gating strategy and baseline lymphocytes for SARS-CoV-2 vaccination study. Related to Figures 1, 2, and 3. (A) Gating strategy for T and B lymphocytes and B cell subsets. (B-D) Baseline lymphocyte subsets are reported for Moderna (red) and Pfizer (blue) recovered (closed circle, solid line) and naïve (open circle, dotted line) subjects over the course of the study. Total CD3+ and CD19+ (B) or CD4+ and CD8+ T cells (C) are reported as percentage of live single cells. B cell subsets including unswitched MBC, switched MBC, and CD27+ and CD27- memory B cells (D) are reported as percentage of live single CD19+ cells. All significance is reported as differences from baseline (T1) using mixed-effects model with Geisser-Greenhouse correction. *p<0.05, **p<0.01



Supplemental Figure 2. MSD-ELICA binding titers for SARS-CoV-2 isotypes and related antigens. Related to Figure 4. (A) IgG, and IgA binding titers over the course of vaccination for HKU1 S, OC43 S, MERS-CoV S, and SARS-CoV-1 S as determined by MSD-ELICA and calculated from reference standard curve; data shown for Moderna (red) and Pfizer (blue) recovered (closed circle, solid line) and naïve (open circle, dotted line), (B) IgG and IgA binding titers against SARS-CoV-2 NP in recovered and naïve individuals over the course of vaccination as determined by MSD-ELICA and calculated from reference standard curve (C) IgM binding titers against S and RBD over the course of vaccination as determined by MSD-ELICA and calculated from reference standard. All significance is reported as differences from baseline (T1) using mixed-effects model with Geisser-Greenhouse correction. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001



Supplemental Figure 3. MSD-ELICA titers to variant RBD and neutralization by vaccine brand. Related to Figure 4. A) IgG binding titers over the course of vaccination for WT, Beta, and Delta RBD as determined by MSD-ELICA and calculated from reference standard. (B) Comparison of neutralization of WT SARS-CoV-2 as measured by in vitro neutralization assay in Moderna v Pfizer in recovered and naïve individuals.



Supplemental Figure 4. RBD-specific antibody depletion by isotype and correlation of anti-Spike titers to RBD-specific neutralization. Related to Figure 6. (A) Fold change in IgM, IgG, and IgA RBD-specific titers post-depletion of RBD-binding antibodies, (B) IgG titers against Spike [WT, B, d] correlated to % reduction of neutralization post RBD-depletion; coefficient of determination (r2) and significance determined from linear regression analysis.

	Recovered (n=40)	Naïve (n=27)
Gender		
Male	19 (47.5%)	11 (40.7%)
Female	21 (52.5%)	16 (59.3%)
Race		
White	35 (87.5%)	16 (59.3%)
Asian	4 (10%)	9 (33.3%)
Black	1 (2.5%)	2 (7.4%)
Age	55 (21-77)	34 (22-64)
Days Post-Symptom Onset (Range)	296 (40-388)	N/A
Pre-Existing Conditions		
Asthma	13 (32.5%)	1 (3.7%)
Hypertension	11 (27.5%)	2 (7.4%)
Anxiety/Depression/ADD	8 (20%)	6 (22.2%)
High Cholesterol	7 (17.5%)	1 (3.7%)
Allergies	5 (12.5%)	4 (14.8%)
Diabetes	5 (12.5%)	2 (7.4%)
Heart Disease	4 (10%)	0 (0%)
Osteoarthritis	4 (10%)	0 (0%)
Thyroid Condition	4 (10%)	2 (7.4%)
Migraines/Chronic Headaches	4 (10%)	1 (3.7%)
Sleep Disorder	4 (10%)	1 (3.7%)
Cancer (Current/Remission)	3 (7.5%)	0 (0%)
HIV-1	0 (0%)	1 (3.7%)
Other	15 (37.5%)	7 (25.9%)
Vaccine Brand		
Moderna	14 (35%)	9 (33.3%)
Pfizer	26 (65%)	18 (66.7%)
Dose 1 Systemic Side Effects		
Fever	12 (30%)	0 (0%)
Headache	10 (25%)	4 (14.8%)
Fatigue	19 (47.5%)	3 (11.1%)
Muscle/Body Aches	14 (35%)	4 (14.8%)
Malaise	9 (22.5%)	5 (18.5%)
Other	9 (22.5%)	1 (3.7%)
Dose 2 Systemic Side Effects		
Fever	15 (37.5%)	8 (29.6%)
Headache	13 (32.5%)	19 (70.4%)
Fatigue	22 (55%)	10 (37%)
Muscle/Body Aches	16 (40%)	10 (37%)
Malaise	3 (7.5%)	4 (14.8%)
Other	15 (37.5%)	10 (37%)

Supplemental Table 1. Demographic information for Emory University SARS-CoV-2 immune surveillance and vaccination studies. Participant age, sex, race, vaccine-type, pre-existing conditions, and systemic side effects are listed. The days between symptom onset and first vaccination dose are listed for the SARS-CoV-2 recovered cohort. Related to STAR Methods subject details.

			Row Statistics									p values: Mixed-Model ANOVA								
			RBD+ MBC			RBD+ IgG+ MBC			RBD+ IgA+ MBC			RBD+ MBC			RBD+ IgG+ MBC			RBD+ IgA+ MBC		
		n	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna
1	Recovered: Pfizer	18	0.084	0.096	0.055	0.050	0.072	0.053	0.013	0.013	0.005	0.9997	0.0034	0.0036	>0.9999	0.0107	0.0058	0.9297	0.9989	0.801
	Recovered: Moderna	13	0.114	0.139	0.099	0.069	0.097	0.078	0.016	0.022	0.015	X	0.0674	0.0467	X	0.0991	0.0725	X	0.4659	0.1880
	Naïve: Pfizer	18	0.021	0.028	0.023	0.011	0.014	0.014	0.005	0.009	0.011	X	X	>0.9999	X	X	>0.9999	X	X	>0.9999
	Naïve: Moderna	9	0.014	0.022	0.027	0.007	0.010	0.012	0.003	0.006	0.008	X	X	X	X	X	X	X	X	X
2	Recovered: Pfizer	17	0.420	0.548	0.413	0.376	0.469	0.360	0.036	0.056	0.063	0.7311	0.0041	0.0035	0.6981	0.0037	0.0034	>0.9999	0.2056	0.2405
	Recovered: Moderna	11	1.010	1.182	0.806	0.959	1.104	0.799	0.063	0.063	0.042	X	0.0333	0.0316	X	0.0457	0.0445	X	0.0437	0.0505
	Naïve: Pfizer	18	0.021	0.033	0.029	0.012	0.016	0.015	0.004	0.007	0.007	X	X	>0.9999	X	X	>0.9999	X	X	>0.9999
	Naïve: Moderna	9	0.019	0.024	0.014	0.008	0.012	0.010	0.005	0.008	0.006	X	X	X	X	X	X	X	X	X
3	Recovered: Pfizer	14	0.660	0.827	0.670	0.589	0.765	0.632	0.021	0.039	0.037	>0.9999	0.0386	0.0351	>0.9999	0.0350	0.0348	>0.9999	0.4436	0.5113
	Recovered: Moderna	7	0.956	0.859	0.553	0.844	0.787	0.548	0.062	0.060	0.041	X	0.3148	0.3014	X	0.3728	0.3716	X	0.5648	0.5787
	Naïve: Pfizer	18	0.052	0.065	0.045	0.034	0.037	0.024	0.009	0.011	0.008	X	X	>0.9999	X	X	>0.9999	X	X	>0.9999
	Naïve: Moderna	9	0.045	0.056	0.029	0.030	0.036	0.020	0.006	0.011	0.009	X	X	X	X	X	X	X	X	X
4	Recovered: Pfizer	16	0.597	0.844	1.058	0.514	0.776	1.008	0.024	0.046	0.064	>0.9999	0.5775	0.9717	>0.9999	0.5958	0.9642	>0.9999	0.9653	>0.9999
	Recovered: Moderna	10	0.605	0.632	0.270	0.565	0.565	0.264	0.028	0.045	0.039	X	0.0121	0.6095	X	0.019	0.5511	X	0.8155	>0.9999
	Naïve: Pfizer	18	0.143	0.157	0.089	0.116	0.127	0.081	0.013	0.015	0.011	X	X	0.9531	X	X	0.9221	X	X	0.9999
	Naïve: Moderna	9	0.223	0.332	0.248	0.179	0.283	0.209	0.018	0.033	0.037	X	X	X	X	X	X	X	X	X
5	Recovered: Pfizer	18	0.365	0.441	0.413	0.309	0.389	0.390	0.021	0.032	0.029	>0.9999	0.0805	0.1115	>0.9999	0.0881	0.1327	>0.9999	0.8159	0.4982
	Recovered: Moderna	13	0.376	0.418	0.216	0.320	0.369	0.201	0.023	0.034	0.032	X	0.0049	0.0072	X	0.0047	0.0077	X	0.9457	0.7878
	Naïve: Pfizer	18	0.079	0.085	0.039	0.052	0.058	0.028	0.013	0.016	0.012	X	X	>0.9999	X	X	0.9996	X	X	>0.9999
	Naïve: Moderna	9	0.116	0.100	0.039	0.083	0.076	0.033	0.011	0.013	0.009	X	X	X	X	X	X	X	X	X
6	Recovered: Pfizer	18	0.201	0.257	0.206	0.164	0.215	0.199	0.024	0.027	0.018	>0.9999	0.2429	0.4533	>0.9999	0.2422	0.6188	>0.9999	>0.9999	0.8701
	Recovered: Moderna	12	0.240	0.259	0.098	0.197	0.215	0.095	0.021	0.026	0.017	X	0.0062	0.0212	X	0.0084	0.0473	X	>0.9999	0.9848
	Naïve: Pfizer	18	0.098	0.105	0.054	0.067	0.069	0.031	0.017	0.020	0.020	X	X	>0.9999	X	X	0.9972	X	X	>0.9999
	Naïve: Moderna	9	0.115	0.121	0.045	0.088	0.094	0.041	0.013	0.016	0.011	X	X	X	X	X	X	X	X	X
7	Recovered: Pfizer	17	0.156	0.220	0.171	0.108	0.170	0.159	0.024	0.030	0.019	0.9979	0.9781	>0.9999	0.9954	0.9516	>0.9999	>0.9999	>0.9999	>0.9999
	Recovered: Moderna	8	0.306	0.326	0.152	0.283	0.270	0.133	0.036	0.040	0.024	X	0.3485	0.9670	X	0.2478	0.8199	X	0.9983	>0.9999
	Naïve: Pfizer	17	0.132	0.140	0.065	0.088	0.093	0.042	0.026	0.025	0.019	X	X	0.9997	X	X	0.9898	X	X	>0.9999
	Naïve: Moderna	8	0.177	0.200	0.107	0.144	0.145	0.075	0.017	0.026	0.024	X	X	X	X	X	X	X	X	X

Supplemental Table 2: Statistics for RBD-specific MBC. Median, mean, standard deviation, and n are listed for data reported in Figure 1. Additionally, comparison between groups at each timepoint is reported, as calculated by mixed-effects model with Geisser-Greenhouse correction. Related to Figure 1.

			Statistics: Figure 3															
			Total Plasmablasts				RBD+ Plasmablasts				RBD+ IgG+ Plasmablasts				RBD+ IgA+ Plasmablasts			
	Timepoint	n	Median	Mean	SD	p value	Median	Mean	SD	p value	Median	Mean	SD	p value	Median	Mean	SD	p value
Naïve: Pfizer	1	18	0.76	0.80	0.55	X	0.44	0.53	0.51	X	0.02	0.13	0.19	X	0.17	0.31	0.58	X
	2	18	2.01	2.45	1.82	0.0010	0.63	1.12	1.03	0.1133	0.21	0.31	0.31	0.0716	0.28	0.64	0.67	0.3600
	3	18	1.03	1.27	1.38	0.3079	0.89	0.91	0.52	0.1141	0.24	0.37	0.42	0.0949	0.46	0.42	0.27	0.8091
	4	18	1.36	1.81	1.51	0.0070	3.66	4.75	6.92	0.0645	1.50	2.02	1.47	0.0003	0.81	2.61	7.18	0.4939
	5	18	0.74	0.97	1.02	0.6068	0.29	0.33	0.32	0.5271	0.04	0.13	0.19	0.9999	0.17	0.19	0.19	0.8148
Naïve: Moderna	1	9	0.96	1.46	1.80	X	0.00	0.20	0.34	X	0.00	0.03	0.09	X	0.00	0.10	0.14	X
	2	9	1.03	2.29	2.55	0.7973	0.39	0.69	0.52	0.0314	0.09	0.14	0.15	0.3203	0.36	0.39	0.32	0.0338
	3	9	0.74	1.42	1.69	0.9973	0.42	0.46	0.18	0.2021	0.10	0.12	0.13	0.4141	0.21	0.22	0.18	0.4046
	4	9	1.01	1.81	1.49	0.9388	1.50	2.40	2.49	0.1008	0.78	1.61	2.07	0.1477	0.37	0.48	0.43	0.1209
	5	9	0.81	1.41	1.47	0.9980	0.26	0.35	0.23	0.7586	0.04	0.08	0.10	0.7978	0.07	0.08	0.08	0.9963
Recovered: Pfizer	1	17	0.61	0.64	0.34	X	0.18	0.34	0.39	X	0.00	0.07	0.13	X	0.11	0.19	0.21	X
	2	15	1.13	1.97	3.13	0.2642	3.17	4.51	3.37	0.0008	1.56	3.58	4.12	0.0137	1.01	1.36	1.26	0.0090
	3	13	0.54	2.03	5.20	0.6945	0.37	0.62	0.67	0.4548	0.09	0.16	0.19	0.5535	0.21	0.34	0.44	0.5652
	4	16	0.74	0.93	0.83	0.3426	0.94	1.90	2.19	0.0432	0.51	1.20	1.95	0.0953	0.44	0.76	1.00	0.1212
	5	17	0.59	1.14	1.84	0.6025	0.45	0.48	0.43	0.2103	0.00	0.09	0.13	0.9509	0.23	0.24	0.21	0.6890
Recovered: Moderna	1	13	1.68	1.53	0.96	X	0.51	0.62	0.52	X	0.04	0.07	0.08	X	0.41	0.44	0.35	X
	2	7	1.38	10.14	22.79	0.6591	2.39	4.22	4.81	0.2149	2.11	2.30	1.78	0.0363	0.66	1.91	3.00	0.5214
	3	7	0.75	1.79	2.11	0.9812	1.24	1.52	1.23	0.2136	0.36	0.42	0.33	0.0612	1.05	1.05	0.69	0.0855
	4	10	1.01	1.18	0.77	0.6721	0.65	1.04	0.94	0.4651	0.34	0.40	0.34	0.0460	0.51	0.53	0.46	0.9458
	5	13	0.85	1.81	2.70	0.9867	0.56	0.66	0.48	0.9997	0.09	0.08	0.10	0.9896	0.28	0.43	0.38	0.9999

Supplemental Table 3. Statistics for total and RBD-specific plasmablasts. Median, mean, standard deviation, and n are listed for data reported in Figure 3. Additionally, comparison between baseline (T1) and each subsequent timepoint is shown for each group, as calculated by mixed-effects model with Geisser-Greenhouse correction. Related to Figure 3.

			Row Statistics						p values: Mixed-Model ANOVA					
			Spike-specific IgG			Spike-specific IgA			Spike-specific IgG			Spike-specific IgA		
		n	Median	Mean	SD	Median	Mean	SD	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna
1	Recovered: Pfizer	18	7743	9101	8824	2349	7800	20304	0.4691	0.0026	0.0028	0.8516	0.4066	0.4105
	Recovered:Moderna	10	11673	22068	26428	2648	3823	3939	X	0.1063	0.1082	X	0.0657	0.0686
	Naïve: Pfizer	18	130	188	182	129	160	108	X	X	0.7393	X	X	0.8898
	Naïve: Moderna	9	167	287	260	146	195	127	X	X	X	X	X	X
2	Recovered: Pfizer	18	154454	190874	174419	27771	73894	117811	0.0115	0.0013	0.0013	0.8036	0.0897	0.1038
	Recovered:Moderna	10	923430	1053904	666025	80558	125837	159372	X	0.0034	0.0034	X	0.1405	0.1492
	Naïve: Pfizer	17 (18)	592	1174	2154	982	3437	4703	X	X	0.9899	X	X	0.9138
	Naïve: Moderna	9 (8)	674	982	1040	893	5437	8150	X	X	X	X	X	X
3	Recovered: Pfizer	15	231553	323662	221920	29026	66425	110165	0.2823	0.0051	0.0035	0.9809	0.4166	0.9939
	Recovered:Moderna	7	665004	772734	581013	25652	52728	59051	X	0.0637	0.0693	X	0.5404	0.999
	Naïve: Pfizer	18	14363	59636	181040	6685	18486	45953	X	X	0.989	X	X	0.4411
	Naïve: Moderna	9	67231	74199	55081	31208	57133	67406	X	X	X	X	X	X
4	Recovered: Pfizer	18	457872	526984	354184	83767	171887	273028	0.272	0.6428	0.995	0.8803	0.7497	0.4205
	Recovered:Moderna	10	608471	896999	554237	54336	114896	134137	X	0.0847	0.2324	X	0.9949	0.2333
	Naïve: Pfizer	18 (16)	243960	370891	434617	81781	103207	94152	X	X	0.8061	X	X	0.1978
	Naïve: Moderna	9	409034	496538	286100	226045	418948	423853	X	X	X	X	X	X
5	Recovered: Pfizer	18	298750	384179	301262	26009	110400	176488	0.6394	0.0858	0.1543	0.5834	0.2419	0.6163
	Recovered:Moderna	10	436800	544695	361154	48460	54145	43764	X	0.0542	0.0725	X	0.3558	>0.9999
	Naïve: Pfizer	18	160622	191030	127502	18675	28246	25446	X	X	0.9736	X	X	0.4906
	Naïve: Moderna	9	197997	210582	104631	25241	55080	51874	X	X	X	X	X	X
6	Recovered: Pfizer	18	238003	365996	412177	37797	111758	194121	>0.9999	0.0259	0.0523	0.6645	0.149	0.2791
	Recovered:Moderna	10	297118	364699	265270	37290	55097	58427	X	0.0221	0.0406	X	0.1258	0.4765
	Naïve: Pfizer	18	41888	57148	34587	5169	8490	13882	X	X	0.1586	X	X	0.2343
	Naïve: Moderna	9	77562	90687	37769	24376	25431	22998	X	X	X	X	X	X
7	Recovered: Pfizer	18	92999	192834	227158	19116	51396	72614	0.9946	0.0224	0.0452	0.852	0.0663	0.0726
	Recovered:Moderna	9	182654	209408	121187	29016	35537	29459	X	0.0065	0.0107	X	0.0583	0.0661
	Naïve: Pfizer	17	13112	19046	12830	3282	5048	8301	X	X	0.4745	X	X	0.9736
	Naïve: Moderna	8	27279	36004	30538	5315	6009	2905	X	X	X	X	X	X

Supplemental Table 4. Statistics for anti-spike antibody across seven timepoints in cohort grouped by past infection status (recovered, naïve) and vaccine brand (Moderna, Pfizer); significance calculated from mixed effects model with Geisser-Greenhouse correction. Related to Figure 4.

			Row Statistics						p values: Mixed-Model ANOVA					
			RBD-specific IgG			RBD-specific IgA			RBD-specific IgG			RBD-specific IgA		
		n	Median	Mean	SD	Median	Mean	SD	Recovered: Moderna	Naïve: Pfizer	Naïve: Moderna	Recovered: Moderna	Naïve: Pfizer	Naïve: Moderna
1	Recovered: Pfizer	18	1970	2898	3325	1048	2115	2422	0.6282	0.0114	0.0154	0.9203	0.0232	0.026
	Recovered:Moderna	10	4030	5832	7196	1697	2922	3620	X	0.1229	0.132	X	0.1651	0.1713
	Naïve: Pfizer	18	88	97	46	215	268	251	X	X	0.5248	X	X	0.992
	Naïve: Moderna	9	81	206	229	205	296	240	X	X	X	X	X	X
2	Recovered: Pfizer	18	63377	87279	87938	10597	29761	40968	0.0877	0.0031	0.0031	0.6538	0.0421	0.1003
	Recovered:Moderna	10	336184	507528	482068	35955	69622	102778	X	0.0367	0.0367	X	0.2248	0.2637
	Naïve: Pfizer	17 (18)	190	271	235	828	1455	1749	X	X	0.6664	X	X	0.6034
	Naïve: Moderna	9 (8)	304	384	241	1801	5116	8036	X	X	X	X	X	X
3	Recovered: Pfizer	15	93676	139404	103322	13947	22310	20778	0.4261	0.0017	0.0053	0.9763	0.9011	0.9431
	Recovered:Moderna	7	212095	309431	267910	15331	28009	34539	X	0.095	0.1144	X	0.8439	0.9993
	Naïve: Pfizer	18	5297	13964	36246	3232	14552	42151	X	X	0.483	X	X	0.7799
	Naïve: Moderna	9	33393	29575	19741	20805	30248	39740	X	X	X	X	X	X
4	Recovered: Pfizer	18	222323	239284	152812	44651	77858	137739	0.3959	0.7021	0.9948	0.9771	0.9995	0.4409
	Recovered:Moderna	10	265166	397243	283078	33210	62127	67514	X	0.172	0.4988	X	0.9767	0.3974
	Naïve: Pfizer	18 (16)	124506	176245	193939	53672	73773	76316	X	X	0.631	X	X	0.4251
	Naïve: Moderna	9	209738	253255	134273	146335	386382	580308	X	X	X	X	X	X
5	Recovered: Pfizer	18	141437	184217	137062	13421	75675	158373	0.7277	0.0646	0.1621	0.6151	0.4693	0.7797
	Recovered:Moderna	10	198762	248148	164835	32584	28989	20302	X	0.0625	0.0984	X	0.667	0.9123
	Naïve: Pfizer	18	80553	91538	57407	14050	19996	19153	X	X	0.9024	X	X	0.5512
	Naïve: Moderna	9	89713	105937	48552	20469	38297	38151	X	X	X	X	X	X
6	Recovered: Pfizer	18	108474	161403	164368	23616	48684	86048	0.9998	0.0136	0.0378	0.8112	0.2016	0.4321
	Recovered:Moderna	10	130464	166045	122732	20332	29310	25490	X	0.0241	0.0508	X	0.0825	0.551
	Naïve: Pfizer	18	20454	25999	16495	3630	6385	10861	X	X	0.0826	X	X	0.3876
	Naïve: Moderna	9	40427	44947	18333	11254	16278	16200	X	X	X	X	X	X
7	Recovered: Pfizer	18	41963	81735	95080	12541	23043	35930	0.9852	0.0211	0.0493	0.9434	0.1444	0.1483
	Recovered:Moderna	9	80828	92250	61090	17213	17876	11239	X	0.0141	0.025	X	0.0216	0.0235
	Naïve: Pfizer	17	5964	8323	5968	2417	3633	5518	X	X	0.3559	X	X	0.9987
	Naïve: Moderna	8	13232	17009	13375	3540	3873	2372	X	X	X	X	X	X

Supplemental Table 5. Statistics for anti-RBD antibody across seven timepoints in cohort grouped by past infection status (recovered, naïve) and vaccine brand (Moderna, Pfizer); significance calculated from mixed effects model with Geisser-Greenhouse correction. Related to Figure 4.

			Row Statistics						p values: Mixed-Model ANOVA					
			NTD-specific IgG			NTD-specific IgA			NTD-specific IgG			NTD-specific IgA		
		n	Median	Mean	SD	Median	Mean	SD	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna	Recovered: Moderna	Naive: Pfizer	Naïve: Moderna
1	Recovered: Pfizer	18	153	156	126	484	2016	5485	0.5325	0.001	0.0011	0.8118	0.4909	0.5415
	Recovered:Moderna	10	212	417	587	323	846	837	X	0.2047	0.2045	X	0.105	0.21
	Naïve: Pfizer	18	5	16	36	93	149	117	X	X	>0.9999	X	X	0.6467
	Naïve: Moderna	9	4	15	22	161	258	262	X	X	X	X	X	X
2	Recovered: Pfizer	18	2706	3978	4053	5352	22391	46733	0.1006	0.0036	0.0035	0.754	0.2433	0.3003
	Recovered:Moderna	10	22292	33769	35425	26936	54827	96775	X	0.0591	0.059	X	0.3489	0.37
	Naïve: Pfizer	17 (18)	13	45	82	483	854	1052	X	X	0.7775	X	X	0.7048
	Naïve: Moderna	9 (8)	22	25	19	571	2278	3623	X	X	X	X	X	X
3	Recovered: Pfizer	15	4976	6953	5732	7833	20474	40864	0.3998	0.0032	0.0074	0.9994	0.3228	0.8638
	Recovered:Moderna	7	14005	19028	18529	5882	18732	29134	X	0.1316	0.1465	X	0.4688	0.9286
	Naïve: Pfizer	18	167	489	1210	1315	1678	2135	X	X	0.5416	X	X	0.1772
	Naïve: Moderna	9	980	1107	1067	6392	11618	12923	X	X	X	X	X	X
4	Recovered: Pfizer	18	8789	11121	9122	19253	44667	89991	0.3484	0.1605	0.8843	0.9794	0.7491	0.3481
	Recovered:Moderna	10	13885	21419	17464	17379	34533	47490	X	0.077	0.2054	X	0.8781	0.2153
	Naïve: Pfizer	18 (16)	3868	5580	6007	17028	22907	17861	X	X	0.5304	X	X	0.1223
	Naïve: Moderna	9	7549	8972	6045	89813	118622	111517	X	X	X	X	X	X
5	Recovered: Pfizer	18	5813	8247	8013	5807	26657	51884	0.6067	0.0528	0.1072	0.7663	0.305	0.5156
	Recovered:Moderna	10	9325	13125	10799	11884	14167	12400	X	0.0597	0.0799	X	0.1434	0.7338
	Naïve: Pfizer	18	2577	2846	1837	3441	4512	4579	X	X	0.8183	X	X	0.5345
	Naïve: Moderna	9	3257	3481	1753	5193	9028	9221	X	X	X	X	X	X
6	Recovered: Pfizer	18	4804	7615	10799	5291	35017	77375	0.9983	0.077	0.1366	0.7752	0.2774	0.3657
	Recovered:Moderna	10	5761	8169	6785	9094	16585	19730	X	0.0347	0.0602	X	0.1249	0.2877
	Naïve: Pfizer	18	830	947	541	856	931	462	X	X	0.2287	X	X	0.261
	Naïve: Moderna	9	1161	1714	1048	2850	4334	5076	X	X	X	X	X	X
7	Recovered: Pfizer	18	1797	3955	5748	4112	14410	25044	0.9578	0.0731	0.1239	0.9255	0.126	0.1379
	Recovered:Moderna	9	3284	4931	4187	7232	10405	8071	X	0.0456	0.066	X	0.0262	0.0306
	Naïve: Pfizer	17	383	369	228	433	546	332	X	X	0.5367	X	X	0.1379
	Naïve: Moderna	8	485	722	697	738	847	289	X	X	X	X	X	X

Supplemental Table 6. Statistics for anti-NTD antibody across seven timepoints in cohort grouped by past infection status (recovered, naïve) and vaccine brand (Moderna, Pfizer); significance calculated from mixed effects model with Geisser-Greenhouse correction. Related to Figure 4.

				Statistics: Recovered vs. Naïve Serology							
				IgG				IgA			
			n	Median	Mean	SD	p value	Median	Mean	SD	p value
Spike-specific	1	Recovered	28	9388	13732	17941	X	2349	6380	16386	X
		Naïve	27	133	221	212	0.0032	129	172	114	0.3276
	2	Recovered	28	283015	499099	586818	X	34141	92445	133596	X
		Naïve	26	603	1107	1823	0.0008	921	4052	5876	0.0114
	3	Recovered	22	343361	466549	418468	X	28459	62067	95550	X
		Naïve	27	21954	64490	149708	0.0016	8656	31368	55887	0.7751
	4	Recovered	28	507754	659132	462575	X	75800	151533	231746	X
		Naïve	27 (25)	335051	412773	390301	0.2335	94503	216874	298915	0.9658
	5	Recovered	28	339704	441506	326736	X	35833	90309	144926	X
		Naïve	27	190196	197547	118685	0.0052	20871	37190	37650	0.3998
	6	Recovered	28	262776	365533	361143	X	37797	91522	160090	X
		Naïve	27	62343	68328	38479	0.0012	6532	14137	18841	0.113
	7	Recovered	27	151403	198359	195758	X	24812	46109	61422	X
		Naïve	25	19894	24473	21140	0.0007	3634	5356	6972	0.0139
RBD-specific	1	Recovered	28	2613	3946	5126	X	1062	2403	2866	X
		Naïve	27	87	134	142	0.0037	214	278	243	0.0038
	2	Recovered	28	123288	237368	352678	X	17161	43997	70400	X
		Naïve	26	240	310	238	0.0098	828	2581	4809	0.0304
	3	Recovered	22	141233	193503	184918	X	14639	24123	25220	X
		Naïve	27	6256	19168	32174	0.0017	4771	19784	41285	0.9994
	4	Recovered	28	255211	295698	217611	X	43361	72240	116291	X
		Naïve	27 (25)	153437	201915	177507	0.4644	68077	186312	373291	0.6893
	5	Recovered	28	145174	207049	147845	X	19875	59001	128253	X
		Naïve	27	89141	96337	54111	0.0051	16370	26096	27658	0.7809
	6	Recovered	28	119127	163061	148448	X	21633	41764	70483	X
		Naïve	27	33037	32315	19083	0.0005	5110	9683	13434	0.1622
	7	Recovered	27	62070	85240	84171	X	13478	21321	29818	X
		Naïve	25	8829	11103	9645	0.0007	2656	3710	4685	0.0365
NTD-specific	1	Recovered	28	175	249	375	X	429	1598	4416	X
		Naïve	27	5	16	32	0.0195	129	185	181	0.53
	2	Recovered	28	6071	14618	25298	X	10481	33975	68901	X
		Naïve	26	14	38	67	0.0351	483	1291	2208	0.122
	3	Recovered	22	5736	10795	12375	X	6857	19920	36830	X
		Naïve	27	267	695	1182	0.0069	1713	4991	8785	0.4248
	4	Recovered	28	10157	14799	13390	X	18010	41048	76650	X
		Naïve	27 (25)	5594	6711	6123	0.0426	21073	57364	80892	0.9859
	5	Recovered	28	6756	9989	9218	X	6431	22196	42229	X
		Naïve	27	2855	3057	1801	0.0036	3785	6018	6677	0.3269
	6	Recovered	28	5324	7813	9426	X	5892	28434	63089	X
		Naïve	27	1019	1202	815	0.0067	1058	2066	3277	0.2254
	7	Recovered	27	3018	4280	5217	X	5638	13075	20829	X
		Naïve	25	406	482	453	0.0058	645	642	344	0.0317

Supplemental Table 7. Statistics for anti-spike, -RBD, and -NTD antibody across seven timepoints in cohort grouped by past infection status (recovered, naïve), regardless of vaccine brand; significance calculated from mixed effects model with Geisser-Greenhouse correction. Related to Figure 4.

CHAPTER 4

DISCUSSION

CONCLUSION

The SARS-CoV-2 pandemic has dominated the global conversation over the last two years with researchers across the world contributing to our understanding of this novel viral threat. Through work with several human cohorts recruited at Emory, we have been able to be a part of this global dialogue, contributing to our understanding of the humoral response to both SARS-CoV-2 infection and mRNA vaccination. In Chapter 2, we provide an in-depth characterization of the B cell response to acute SARS-CoV-2 infection in patients who developed severe COVID-19. Despite the severity of disease, we observed that individuals were able to quickly mount robust cellular and serological responses specific to SARS-CoV-2 and that the neutralizing serological response was dominated by antibodies specific to the RBD on spike. These observations have now been supported by other human cohort studies and provide the foundation for the importance of a protective, functional of B cell response in the context of SARS-CoV-2. However, several outstanding questions remain concerning acute infection, especially in those that develop severe disease. Given that severe COVID-19 can induce broad immunopathology, there is a concern that B cells could be contributing to disease through the potential activation of self-reactive B cells in this hyperactive immune environment. We have begun to explore this question using both plasma and monoclonal antibodies derived from plasmablasts from the above cohort.

In Chapter 3, we sought to characterize both the short- and long-term kinetics of B cell responses to mRNA vaccination in both those with and without previous infection with SARS-CoV-2. We observed a strong induction of cellular and serological responses in both exposure groups with individuals recovered from SARS-CoV-2 having more durable responses that exhibited greater breadth than their naïve counterparts. We additionally confirmed that, similar to

our study of acute infection, the neutralizing serological responses induced by vaccination relied on RBD-specific antibodies, although naïve Moderna-vaccinated individuals had a lesser reliance on this epitope specificity. Given the similarities between the Moderna and Pfizer mRNA vaccines, this result was unexpected, and we are pursuing additional repertoire analyses to explore the difference between these two vaccine brands in addition to differences between naïve and recovered individuals. Additionally, after the conclusion of our vaccine study, the highly mutated and rapidly spreading Omicron variant emerged, calling into question the efficacy of pre-existing immune responses induced in the context of infection and vaccination.

FUTURE CONSIDERATIONS

Autoimmunity and COVID-19

In Chapter 2, we provided an initial characterization of the humoral response in individuals with severe COVID-19. Since the time of publication, significant work has been conducted in the field to further characterize the antibodies induced by infection, and a review of this work is included in Chapter 1. However, a component of this response that continues to merit ongoing investigation is the extrafollicular response to infection compromised of both the expected expansion of ASCs and the increased detection of atypical B cells. We observed plasmablast expansions in the majority of our cohort and sought to further explore this response through the generation of monoclonal antibodies. We performed single-cell sorting of total plasmablasts on four individuals who had robust titers of both RBD-binding and neutralizing antibodies (Figure 1A). This experimental strategy has been applied in other viral infection and vaccination settings and has generated large numbers of antigen-specific antibodies (188, 298). However, monoclonal antibodies derived from these individuals proved, on the whole, to be non-specific to the immunodominant SARS-CoV-2 antigens (S, RBD, N) (Figure 1B). Out of 117 monoclonal antibodies generated, seven antibodies bound to the S protein and only 1 antibody bound to RBD (Figure 1B).

To further investigate the antigen specificity of these antibodies, we screened 75 antibodies using a high-throughput viral and auto-antigen array previously used to screen plasma from COVID-19 patients (329). While previous studies have identified antibodies specific to endemic coronaviruses (191), we did not observe any reactivity between endemic coronavirus S proteins and our antibody library (Figure 1C). While the majority of the 70 non-S reactive antibodies did not bind to antigens included in the microarray, we did observe that 13 of these

antibodies bound to internal SARS-CoV-2 proteins, specifically the 3C-like papain-like proteinases (Figure 1C). Four of these proteinase-binding antibodies also significantly bound to two autoantigens, specifically the complement protein C1q (n=3) and U1 snRNPA (n=1) (Figure 1C). The apparent dual reactivity of these antibodies suggests that molecular mimicry between these internal viral proteins and select autoantigens could exist. Experiments comparing the structure and binding of these antibodies to both viral and host antigens are necessary to further determine whether this mechanism is at play. Additional experiments are also needed to determine whether these antibodies have a potentially pathogenic role in aggravating disease and could be conducted using either human or mouse-adapted SARS-CoV-2 in mouse models (150, 330). In addition to the panel of antibodies, we screened plasma from another nine acutely infected individuals to determine whether autoantibodies could be detected at the plasma level in other individuals within the cohort (Figure 1D). Three of these individuals were positive for autoantibodies, two for the thyroid-associated protein, TPO, and 1 for the immune-associated protein, BPI (Figure 1D). While we cannot rule out that these antibodies were circulating prior to these individuals contracting SARS-CoV-2, it is interesting to note that none of these individuals reported pre-existing conditions that would suggest the presence of these autoantibodies. Additional analyses of these individuals, specifically the generation of plasmablast-derived antibodies, could determine if plasmablasts activated in SARS-CoV-2 infection are the source of these autoantibodies.

Autoantibody production after viral infection has been previously described, such as in cases of Guillain-Barré syndrome after Zika infection during the 2015-2016 epidemic (331), and evidence for a relationship between COVID-19 and autoantibodies has been mounting within the field. While the evidence that autoantibodies can be drivers of severe disease in SARS-CoV-2

infected patients is conflicting (120, 332), two novel studies using high-throughput antigen screening methods have revealed the potential for SARS-CoV-2 infection to drive the *de novo* generation of autoantibodies (329, 333). In the first, 147 plasma samples from COVID-19 patients were screened using a combined viral- and autoantigen array system (329). Approximately 50% of individuals screened were positive for autoantibodies, which were targeted against a wide array of both common and rare autoantigens (329). In a subset of individuals where longitudinal samples were available, autoantibodies were seen to increase over disease progression, suggesting potential *de novo* induction of these antibodies during the humoral response to infection (329). Indeed, autoantibody titers in these individuals correlated with the rise of SARS-CoV-2 related antibodies, further strengthening the link between the two populations (329). A second study using a yeast library approach to screen individuals with COVID-19 for autoantigen reactivity found similar results (333). While a subset of individuals had high autoantibody titers near infection onset, another group had a kinetic pattern that suggested the *de novo* generation of these autoantibodies during SARS-CoV-2 infection (333). It is interesting to analyze the screening results of our plasmablast-derived monoclonal antibodies in parallel with these results as the presence of these potential autoantibody-producing plasmablasts lends additional support for the theory that SARS-CoV-2 infection can lead to the development of autoimmune disorders. These findings are also supported by the increasing number of clinical case reports on the onset of autoimmune disorders after recovery from COVID-19 (334).

Influences on B Cell Repertoire

In Chapter 3, we sought to provide an in-depth characterization of the vaccine-induced humoral immune response in both individuals recovered from and naïve to SARS-CoV-2. While

we observed reductions in neutralizing antibody titers in both the context of SARS-CoV-2 variant challenge and over time, our results indicated that vaccination was able to elicit a robust humoral immune response in individuals with and without pre-existing immunity. However, pre-existing immunity clearly influenced individual's vaccine responses and, while we provided a detailed account of the quantitative differences in cellular and serological immunity, clear qualitative differences were also observed in terms of variant neutralization. Whether these differences are being driven by a differential antibody repertoire induced between infection and vaccination or simply the difference between vaccination as a secondary versus primary exposure is as yet unclear. We also noted that vaccine brand could play a role in generating differential antibody repertoires as evidenced by a greater reliance on RBD-binding antibodies in Pfizer vs Moderna vaccinated individuals in our naïve cohort.

To further explore this point, we conducted studies into the repertoire of naïve (n=8) and recovered (n=7) vaccinated individuals using a 10x-based sequencing approach. Spike-specific MBCs were sorted from individuals 1 month after vaccination, and VDJ libraries were generated for each individual with a median of 356 (range 16-1,1153) unique antibody sequences per individual. Two recovered individuals with less than 50 sequences were excluded from the final V_H and isotype gene usage analysis but were included in mutation load and public clonotype analyses. V_H gene usage was similar between recovered and naïve individuals with V_H 3 family genes (3-30, 3-23, 3-33) overrepresented into both groups (Figure 2A). Additional V_H 1 (1-69D) and V_H 4 (4-31, 4-39, 4-59) were also highly represented in both groups (Figure 2A). Isotype usage was similar between recovered and naïve individuals with the majority of antibodies belonging to the IgG1 subclass followed by a smaller percentage of IgGA1 and IgG2 antibodies (Figure 2A). Clonality analysis within individuals in both groups revealed very similar patterns

of clonality; examples from each group are shown in Figure 2C. In both naïve and recovered individuals, very few clonal groups were identified, and these clonal groups were not heavily expanded, suggesting that the S-specific antibody response relies on an extremely diverse B cell pool regardless of pre-existing immunity (Figure 2C). Comparisons of V_H gene somatic hypermutation reveal similar patterns between both groups, although slightly higher levels of somatic hypermutation were detected in antibodies from naïve individuals (Figure 2D). These results provide initial evidence of the striking similarity of the antibody repertoire generated in these two groups although we note that these conclusions are limited given the small sample size.

In addition to comparing the effect of pre-existing immunity on the antibody repertoire, we were also able to compare the effects of vaccine brand with the naïve individuals assayed (n=4 for Moderna/Pfizer). Much like the V_H gene and isotype analysis in recovered and naïve vaccinated individuals, we observed extremely similar patterns of gene usage in both Moderna and Pfizer-vaccinated individuals (Figure 2B). We also did not observe any major differences in clonality between the two groups (Figure 2C). Interestingly, we found that individuals vaccinated with Pfizer appear to have more antibodies with higher levels of somatic hypermutation than their Moderna-vaccinated counterparts (Figure 2D). While this difference is slight, it potentially suggests that vaccination with Moderna recruits a greater number of naïve B cells while Pfizer-vaccination is more likely to drawn in pre-existing clones. To further evaluate this claim, monoclonal antibodies generated from both of these groups with both low and high SHM signatures could be compared for cross-reactivity to endemic coronaviral S proteins and additional functional analyses.

Given the magnitude of B and T cell diversity within a single individual, it is somewhat impossible to think that nearly identical clones of these cells could be shared between individuals

in the general population. However, studies conducted with large-scale sequencing methods are becoming more readily available and have found that select antibody clones appear to overlap between individuals exposed to certain pathogens. Understanding the relevance of these clones, termed public clonotypes, is an area of active investigation, and the recent surge of repertoire analyses of SARS-CoV-2 infection by both single cell cloning and 10x sequencing methods have allowed an exploration of this phenomenon on an unprecedented scale. Repertoire analysis of SARS-CoV-2 infected individuals have found RBD-specific clonotypes that are shared between individuals (156) and that a small subset of individuals even contain clones previously characterized in SARS-CoV-1 infection (195). We sought to explore whether antibody clones were shared between recovered and naïve individuals within our vaccinated cohort. Using parameters previously published by other groups (335), we compared the antibody repertoires between individuals and identified shared clones as those with the same V_H and $V_{K/L}$ gene usage and same length of CDR3 with 70% sequence homology. We identified 22 public clonotypes, 6 of which were shared between more than two individuals within the study (Figure 2D). Interestingly, we found that a majority of the public clonotypes found were shared between recovered and naïve vaccinated groups which further supports that vaccination is able to elicit similar responses in both recovered and naïve individuals (Figure 2D). While this sequencing analysis provides initial evidence of a similar antibody repertoire between recovered and naïve individuals, additional experimentation including the functional analysis of public and private clonotypes from these individuals is needed to provide further evidence for this claim.

OUR PANDEMIC FUTURE

Emergence & Escape of Omicron

We found that naïve vaccinated individuals had a significant reduction in neutralization against the beta and delta variants as compared to their recovered counterparts one month after vaccination, indicating the naïve vaccinated individuals could be more susceptible to emerging SARS-CoV-2 variants. While the portion of the population with infection- or vaccine-induced immunity is growing, SARS-CoV-2's continued global circulation threatens the emergence of additional variants able to strongly escape pre-existing immunity. This threat was fully realized in November 2021, almost two years into the pandemic, with the emergence and rapid spread of the Omicron (B.1.1.529) variant (336). Emerging out of South Africa, Omicron was recognized by global public health organizations as a variant of concern within a week of its discovery and had spread to a number of countries within the month (336). The rise of this variant was particularly concerning to scientists as it contained over 30 mutations in the spike protein (336). Evolutionary analyses of the variant confirmed that it had arisen separately from other circulating variants, and its origin is still a matter of debate, with some hypothesizing its emergence from an immunocompromised individual with persistent SARS-CoV-2 replication (336-338). Previous reports characterizing the evolution of SARS-CoV-2 within immunocompromised individuals provides tangential support to this theory although further investigation is needed (337).

Some of the mutations found in Omicron's RBD, such as K417N, E484A, and N501Y, had been characterized in previous variants and known to cause enhanced viral transmission and immune escape as discussed in Chapter 1. Several mutations are unique to Omicron, and the predicted combinatorial effect of these mutations, which are spread throughout the NTD, RBD,

and fusion peptide, is severely reduced immunity and potentially increased virulence (336). Studies of ACE2-Omicron RBD binding report slight increases in receptor affinity in addition to novel affinity to the mouse ACE2 receptor (339). However, these increases in affinity are much less than predicted given the positive mutations to three key residues (S477/E484/N501), and the attenuated increase in receptor affinity is likely due to the combinatorial effects of all of the mutations at this site (338). Additional conformational changes to the RBD are predicted based on the number of mutations (338) although the potential effects of this change remain to be explored.

Rapid studies of Omicron binding and neutralization have demonstrated that protective immunity is several reduced against this variant. Plasma from recovered individuals consistently demonstrates little to no neutralizing ability against Omicron (339, 340) regardless of previous infecting variant (338), and this decrease in neutralization is accompanied by a significant decrease in RBD binding (340). Individuals vaccinated with either mRNA (212, 339, 340) or AstraZeneca's adenoviral vaccine (212, 339) also display severely reduced neutralization 1 month after vaccination and little to no neutralizing ability after 5-6 months (212,341). While a reduction in neutralization as compared to wild-type is still observed, individuals with immunity generated from the following combinations retain neutralization towards Omicron: individuals receiving three mRNA (338, 340, 342) or adenoviral (338, 342) vaccine doses, individuals both recovered and receiving two vaccine doses (338, 340), and individuals both recovered and receiving three vaccine doses (340). Interestingly, in one study comparing two to three vaccines doses, plasma neutralization of Omicron only correlated with wild-type neutralization in the boosted individuals, suggesting that the booster dose is able to elicit cross-protective antibodies types not dominant in initial vaccination (342). While continued studies will be required to

assess the durability and protective nature of these neutralizing responses, initial evidence strongly supports the need for combinatorial immunity derived from either multiple vaccine doses or infections.

With direct treatments of COVID-19 lagging behind vaccination efforts, any disruption in the current monoclonal antibody therapies approved and in trials would be devastating. Initial studies of approved monoclonal antibodies against Omicron consistently observe a complete ablation of neutralizing activity for the combinatorial therapies from Lily and Regeneron and a significant decrease in neutralization for the majority of other approved and candidate monoclonal therapies (212, 338, 343). However, one monoclonal antibody, Sotrovimab from GSK, retains significant neutralizing activity against Omicron (212, 338, 339). This monoclonal antibody, derived from a SARS-CoV-1 infected individual (173), targets a unique, cross-reactive epitope within the RBD (343). Preliminary work characterizing the large pools of antibodies derived from SARS-CoV-2 infected individuals demonstrates an overarching loss or reduction in neutralization potential due to the extensive changes to the RBD and NTD regions in Omicron (339, 343). While there is a loss of neutralizing activity of RBD antibodies that target epitopes that directly interfere with ACE2 binding, the small subset of RBD antibodies targeting "cryptic" epitopes appear to retain neutralization against Omicron (338, 339, 343). Taken together, these findings illustrate not only the need to find more robust COVID-19 treatment alternatives but also the importance of generating broadly cross-reactive immunity to mutable circulating pathogens.

"Future Vaccines" : Combatting Variants Before They Arise

The efficacy of booster vaccines to increase neutralizing antibody titers against the rapidly spreading Omicron variant is promising. However, the deployment of this strategy was

not enough to halt the rapid rise in global cases and, with them, increases in hospitalizations and mortality. The idea to generate cross-protective immunity to a pathogen and its many serotypes and variants is not new. Indeed, the use of multivalent vaccines is now common strategy for both viral (344) and bacterial (345) pathogens. Emerging from this field is the development of vaccine candidates that can not only protect against multiple known variants but also generate cross-protection against emerging viral variants or close family members. Homotypic and mosaic nanoparticle vaccines were initially explored in the context of influenza as rapidly mutating influenza strains continue to pose a challenge for the traditional vaccine strategies deployed. A mosaic nanoparticle containing eight RBDs from various influenza strains was found to generate greater neutralizing breadth than traditional trivalent vaccines in a mouse model of influenza (346). This vaccine-induced immunity was not found to be diminished in mice that had previous exposures to influenza, and researchers additionally detected antigen-specific B cells that were able to interact with multiple heterotypic RBDs (346). Building off of these findings in influenza, two groups have pursued the use of these multimeric nanoparticles in the context of SARS-CoV-2 with promising initial results. One group, using ferritin-based homotypic nanoparticles linked to either SARS-CoV-2 spike or RBD, found that these nanoparticles consistently induced neutralizing immunity not only against SARS-CoV-2 but also variants containing K417, E484, and N501 mutations and SARS-CoV-1 in a mouse model (347). Another group used a mosaic nanoparticle approach to express 4-8 RBDs from divergent coronaviruses in an attempt to not only elicit protection against SARS-CoV-2 but to also induce broad neutralizing responses against other coronaviruses with the potential for spillover into the human population (348). These mosaic nanoparticles were able to elicit not only neutralizing antibodies against the viral RBDs linked to the nanoparticle, but plasma from these vaccinated mice was also able to

neutralize coronaviruses whose RBDs were not included in the vaccine (348). Similar to the mosaic influenza vaccine, B cells able to recognize coronaviral RBDs that were only 70% similar were also detected in these animals (348). While still in the initial pre-clinical and clinical stages, the potential to design vaccines that are able to elicit broadly neutralizing responses is an area of great interest especially as the risk for both the emergence of additional variants and pathogens remains high.

SUMMARY

The rapid spread of SARS-CoV-2 through the global community especially through populations that remain unvaccinated either by choice or due to lack of resources is an ongoing public health concern. While the scientific community has exponentially increased our understanding of this novel pathogen and its disease phenotype, efforts are still needed to elucidate the long-term impacts of COVID-19 and the potential role of viral-induced autoimmunity. Additional efforts are also needed in characterizing the protective immune correlates of vaccination and whether qualitative differences in infection and vaccine-induced immunity effect protection. Finally, advances in vaccine development are urgently needed to create vaccine strategies that elicit broader protective immunity in order to continue to prevent not only the spread of SARS-CoV-2 variants, but also to provide protection against the next pathogen to inevitably spillover into the human population.

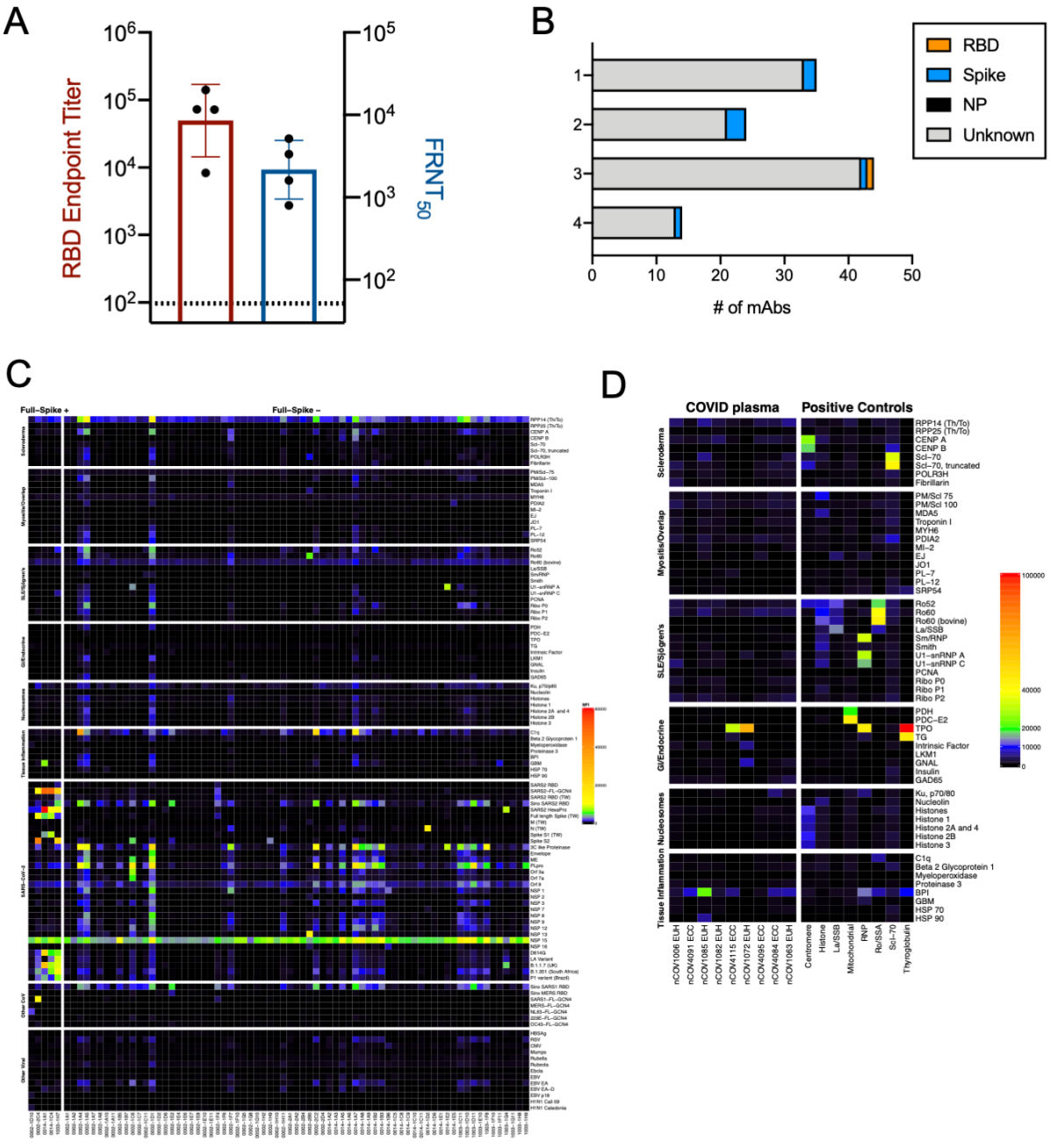

A
RBD Endpoint Titer
FRNT50
B
RBD
Spike
NP
Unknown
# of mAbs
C
Full–Spike +
Full–Spike –
D
COVID plasma
Positive Controls
Scleroderma
Myositis/Overlap
SLE/Sjogren's
GI/Endocrine
Nucleosomes
Tissue Inflammation
Centromere
Histone
La/SSB
Mitochondrial
RNP
Ro/SSA
Scl–70
Thyroglobulin

Figure 1. Majority of plasmablasts induced in severe COVID-19 are of unknown antigen-specificity. A) RBD-binding and neutralization titers for individuals (n=4) from which plasmablasts were sorted and cloned. B) Antigen specificities of plasmablast-derived antibodies based on ELISA screening with recombinant SARS-CoV-2 antigens. C) Heat map of auto- and viral antigen screen of 75 plasmablast-derived antibodies. D) Heat map of autoantigen screen of plasma samples from nine patients with severe COVID-19

*Plasma and antibodies initially characterized by Kauffman, R.C.., Mantus, G., Norwood, C., and Nyhoff, L.E. of the Wrammert Lab and the Suthar Lab at Emory University; microarray screening was conducted by the Utz Lab at Stanford University

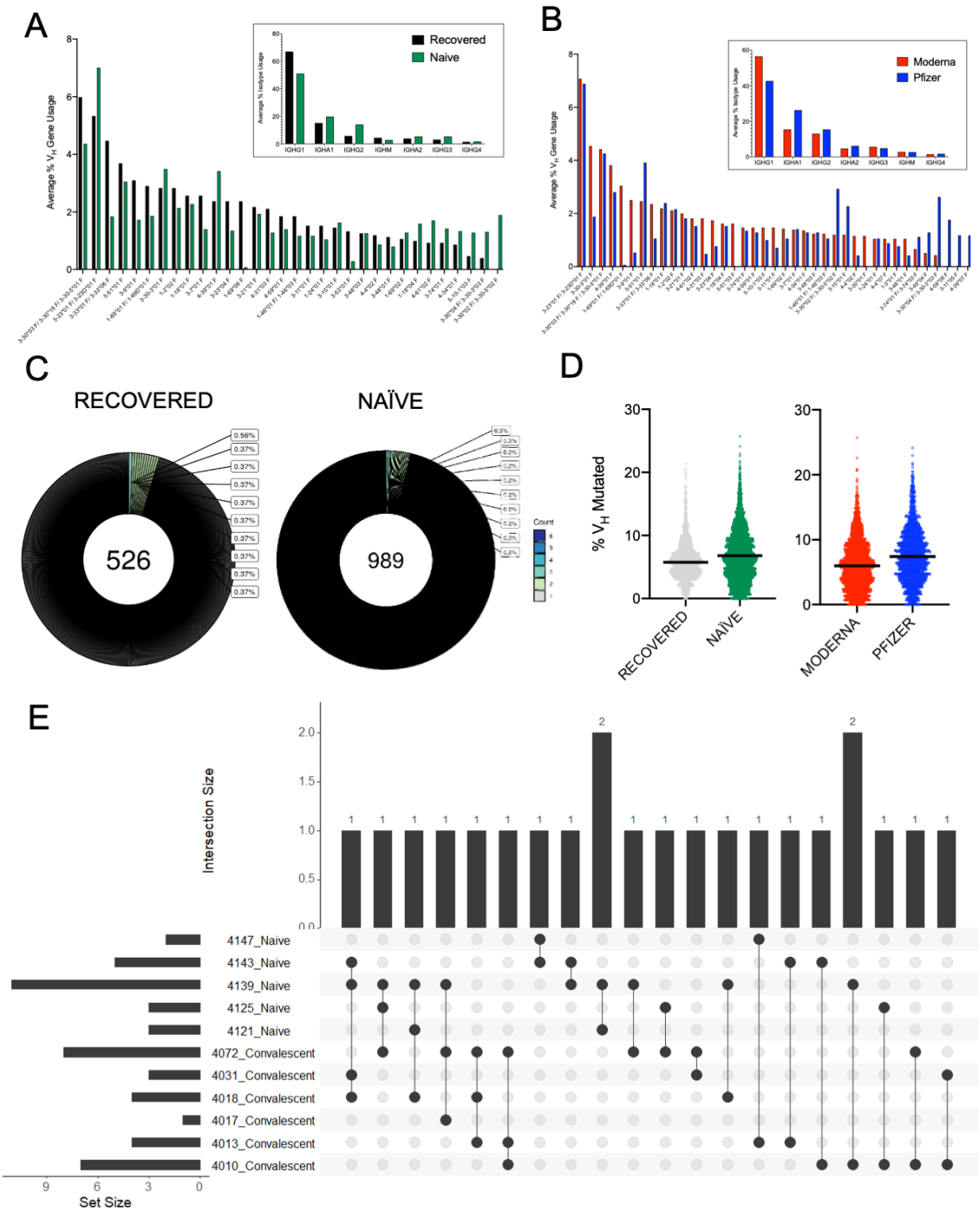

A
Recovered
Naive
Average % V_H Gene Usage
Average % Isotype Usage
IGHG1 IGHA1 IGHG2 IGHM IGHA2 IGHG3 IGHG4
B
Moderna
Pfizer
Average % V_H Gene Usage
Average % Isotype Usage
IGHG1 IGHA1 IGHG2 IGHA2 IGHG3 IGHM IGHG4
C
RECOVERED
NAÏVE
526
989
Count
D
% V_H Mutated
RECOVERED
NAÏVE
MODERNA
PFIZER
E
Intersection Size
Set Size
4147_Naive
4143_Naive
4139_Naive
4125_Naive
4121_Naive
4072_Convalescent
4031_Convalescent
4018_Convalescent
4017_Convalescent
4013_Convalescent
4010_Convalescent

Figure 2. Antibody repertoires between recovered and vaccinated individuals are similar and share several public antibody clones. V_H and isotype gene usage in A) recovered and naïve individuals and B) naïve individuals vaccinated with Moderna and Pfizer; V_H and isotypes shown which accounted for greater than or equal to 1% of the total gene usage in each group C) Examples of clonal analysis in a recovered and vaccinated individual. D) V_H mutations in recovered and naïve (Moderna and Pfizer) individuals. E) Public clonotype sharing between recovered and naïve vaccinated individuals with set size indicating the number of clones that account for public clonotypes in each individual and intersection size denoting the number of clones included in each individual public clonotype.

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Illumina, GlaxoSmithKline, MSD, and Roche-Ventana, unrelated to this Correspondence; and stock options from Apogen Biotech, Epic Biosciences, GRAIL, and Achilles Therapeutics, unrelated to this Correspondence. All other authors declare no competing interests. ECW, MW, SG, and DLVB contributed equally. GKa, CSw, SGan, and DLVB are joint senior authors. RB and DLVB are members of the Genotype-to-Phenotype UK National Virology Consortium. Funding details and acknowledgments can be found in the appendix. All data (anonymised) and full R code to produce all figures and statistical analysis presented in this Correspondence are available online on Github.

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