SARS-CoV-2 Humoral and Cell Mediated Immune Dynamics

by

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B.S., University of Pittsburgh, 2021

Submitted to the Graduate Faculty of the

Department of Infectious Diseases and Microbiology

School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Public Health

University of Pittsburgh

2022

UNIVERSITY OF PITTSBURGH

SCHOOL OF PUBLIC HEALTH

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on

April 29, 2022

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Abstract

Background: The ongoing Covid-19 pandemic has been characterized by waves of new cases, vaccinations, and variants. Understanding the impact of these cumulative exposures on immunity to circulating SARS-CoV-2 is needed to improve public health recommendations. Global vaccination efforts must utilize newly available information about SARS-CoV-2 correlates of immunity to improve efficacy and efficiency.

Objective: This essay aims to describe what is known and unknown about the adaptive immune response to SARS-CoV-2 infection and vaccination.

Review: I analyzed the dynamics of antibody, B cell, and T cell responses to various Covid-19 exposures and characterized their role as correlates of immune protection against SARS-CoV-2 infection. I describe potential flaws in our assessment of vaccine efficacy and propose areas of interest for future research

Conclusion: T cell responses to SARS-CoV-2 better describe the effector functions of the host immune response than antibody titers. Vaccines effectively prevent against symptomatic and severe Covid-19 disease. Future research is needed to accurately describe how antibody, B cell, and T cell responses serve as a correlate of immunity

Public Health Significance: The dynamics described in this paper suggest that the basis of current vaccine recommendations may be improved. Elucidating these characteristics of

immunity will inform vaccination efforts and improve strategies to control the spread of Covid-19.

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Preface

I would like to thank Robbie Mailliar for his guidance during my time at Pitt Public Health. His unrelenting excitement about immunology and research is contagious. I extend my thanks to all my colleagues at the School of Public for enriching my learning experience. I am thankful I was able to navigate and endure the Covid-19 pandemic with you all. Thanks to my friends and family for their abundant support. And special thanks to my loving dad, who teased me for applying to this degree program pre-pandemic when there were "no real public health opportunities"— I can finally say that you were wrong.

1.0 Introduction

Covid-19 Pandemic

Since December of 2019, there have been over 509 million confirmed cases of Covid-19 and more than 6 million deaths attributed to the virus globally.[1] As the Covid-19 pandemic continues to impact the daily lives of most individuals, the world relies on public health authorities for guidance. Rapidly emerging information about this novel virus has improved strategies employed to control the virus. Public health measures have since been aided by the distribution of Covid-19 vaccines. New evidence about the immunology and pathogenesis of the virus provide insight on how prior infection and vaccination impact individual immunity to Covid-19. However, understanding how these different combinations of exposures provide protection against new variants is critical to providing efficient and effective vaccine recommendations. As we enter the third year of living with Covid-19, following the largest wave of cases yet, we must critically assess our understanding of the virus and prioritize areas for future research.

SAR-CoV-2 Infection

Covid-19 infection in humans is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the *Coronaviridae* family, and is transmitted through respiratory droplets of infected individuals.[2,3] SARS-CoV-2 consists of four structural proteins: spike (S), nucleocapsid (N), membrane (M), and envelope (E)[2] (*Fig. 1A).* Infection is established in respiratory mucosa when the S protein binds to the host's Angiotensin Converting Enzyme 2 (ACE 2) and enters the cells.[2] The S protein is cleaved into two subunits, S1 and S2 by the host.[2] The S1 subunit facilitates viral entry via the receptor binding domain (RBD) *figure 1b.*[2,4,5] The

pathogen's pandemic status is attributed to novel differences in the SARS-CoV-2 RBD from previously circulating coronaviruses.[6]

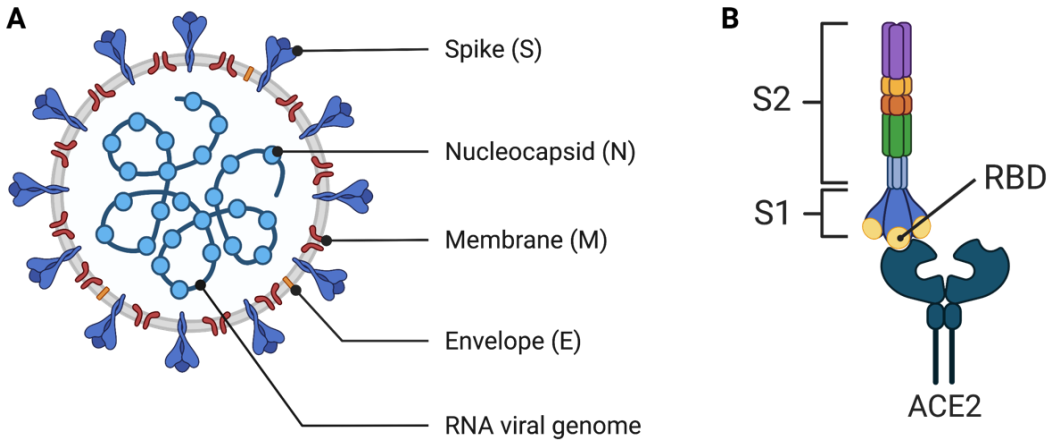

Figure 1 ***SARS-CoV-2 Structure*** A) SARS-CoV-2 proteins encase the viral genome B) S protein subunits and RBD bind to host ACE2 receptor

The major components of the adaptive immune response to SARS-CoV-2 infection include antibodies, B cells, and T cells.[3-9] Dynamics of the humoral and cell-mediated immune responses to SARS-CoV-2 are variable across individuals, making them difficult to characterize.[10,11] However, a broad and coordinated immune response provides the strongest protection against Covid-19.[8] A more comprehensive understanding of adaptive immune dynamics may help.

Covid-19 Vaccines

The unprecedented development and distribution of vaccines against novel SARS-CoV-2 within the first year of the pandemic provided promise of normal life. The Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) vaccines are the first publicly available mRNA

vaccines, and large-scale evaluations of their efficacy are ongoing.[12] In the United States, 92% of fully vaccinated individuals have been vaccinated with an available mRNA vaccine.[13] Both the Pfizer-BioNTech and Moderna vaccines deliver mRNA encoding the full-length S protein and stimulate an immune response comparable to that elicited during natural SARS-COV-2 infection.[12,14,15] Establishing a protective antibody response to the S protein was a primary target of vaccine development.[8] Vaccine efficacy has historically been evaluated by neutralizing antibody responses because they provide immediate protection against infection upon exposure.[4] Emerging SARS-CoV-2 variants and declining antibody titers in vaccinees have raised concerns about the durability of vaccine induced immunity.[16-19] Evidence suggests that cell-mediated immunity may provide additional protection against severe disease.[7,17,21,23] A better understanding of how adaptive immune components are activated during SARS-CoV-2 exposure following infection or vaccination is needed.[3,17,20,6]

1.1 Essay Aim

In this essay I describe what is known about the adaptive immune response to SARS-CoV-2 exposure, both naturally and vaccine mediated. I similarly describe gaps in our understanding and highlight priority areas for future research. The larger study objective is to develop an adaptable framework for elucidating varied immune dynamics to SARS-CoV-2. As the Covid-19 pandemic continues to evolve, these characterizations may improve efficacy of public health measures and vaccination strategies.

2.0 Review

Antibodies

Neutralizing antibodies, which block viral entry and prevent infection, are the first line of defense against pathogen encounters. Antigen specific neutralizing antibodies targeting the RBD of the S protein inhibit SARS-CoV-2 bind to the host's ACE2 receptor on the respiratory mucosal surfaces.[3,4,7] Following a natural SARS-CoV-2 infection, 90% of neutralizing antibodies detected in serum target the S protein and come in IgM, IgA, and IgG immunoglobulin isotypes.[3] Maximum neutralizing activity is achieved when all three types of antibodies are present.[24] IgM antibodies produced during early infection are detectable at day 4 post symptom onset but typically diminish by day 20.[24] IgA antibodies are detected between day 6 and 10 post symptom onset, peak around day 20, and rapidly decline around day 60.[24] IgG antibodies develop 10-14 days after symptoms and dominate the immunoglobulin profile of convalescent sera following viral clearance.[24] The detection and durability of antibody isotypes following SARS-CoV-2 infection varies by individual host factors and testing methods.[20]

Serum levels of IgA are markedly high in individuals with severe disease and can be low or undetectable in individuals who experience mild or asymptomatic infections.[9,21,24,25] Serum from blood draws may be conveniently utilized to assess an individual's antibody responses, however, the differences of the immune response in mucosal tissue and blood must be considered.[3] Antibodies residing in mucosal membranes, which is typically dominated by IgA, represent different isotype profiles from the serum.[22] Studies comparing antibody profiles by sample strategy found that immunoglobulin stratified antibody titers in saliva and bronchoalveolar lavage (BAL) samples were not significantly correlated with serum samples, particularly for IgA

isotypes.[26] IgA may also be detected in saliva or nasal swabs several days after declining in serum.[24,26] This suggests that the SARS-CoV-2 host response has specific mucosal characteristics that may not develop systemically or be represented in serum. Interestingly, a study of immunoglobulin specific neutralizing capacity found monomeric serum IgG to be twice as potent as its IgA counterpart, but the dimeric form of IgA secreted in the mucosa has 15 times the neutralizing potency of monomeric IgA.[27] This unique investigation of specific IgG and IgA forms may explain contrasting results of previous studies on the relative neutralization potency of serum IgG and IgA. Together these finding suggest that antigen specific neutralizing IgA effectively prevents SARS-CoV-2 infection in the mucosal tissue.

There appears to be a transition from IgA dominant antibody profile to IgG dominant profile from the nasal compartments to the lungs (Fig. 2). IgA is more prevalent in saliva samples from the upper respiratory tract than BAL samples of the lower respiratory tract and even more so than serum samples.[4,26] Though neutralization is critical for preventing establishment of infection, antibodies facilitate viral elimination through methods such as antibody dependent cytotoxicity, phagocytosis, and complement activation.[16,25] These functions are most often attributed to IgG antibodies.[25] Successful use of convalescent plasma therapy in Covid-19 treatments support the idea that IgG antibodies in the plasma promote viral elimination.[9,24] Though IgA antibodies are critical during viral encounter in the upper respiratory mucosal surface, IgG antibodies residing deep in the respiratory tract and blood are likely essential for managing systemic viral dissemination.

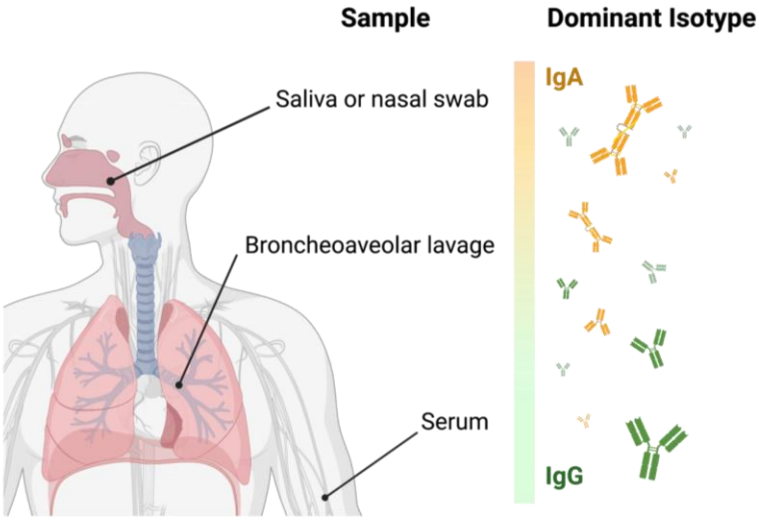

Figure 2. ***Antibody distribution*** Gradient of antibody types present in Samples taken from the upper respiratory tract to the blood stream

Antibodies produced during initial infection prevent viral dissemination and promote viral elimination but circulating neutralizing antibodies present during secondary exposure are necessary to inhibit viral entry of the host cell (Source). The Pfizer BioNTech and Moderna mRNA vaccines induce similar high affinity antibody response against the S protein comparable to that elicited during natural infection.[15,17,28] When individuals with prior SARS-CoV-2 infections were vaccinated with two doses of an mRNA vaccine, antibody titers were boosted after the first dose but not the second dose.[17] SARS-CoV-2 naïve individuals required two doses to achieve similar titers but responses were more variable than the exposed group.[17] Vaccines induce a higher IgG to IgA antibody profile than natural infections.[28] Low antibody titers in nasal swab compared to serum samples in unexposed vaccinated individuals suggest the vaccine does not produce antibodies at mucosal surfaces and do not elicit a protective humoral response.[18] Vaccination after exposure boosts mucosal antibody titers and provides better response than vaccination alone.[17,18]

Efficacy evaluations have mostly utilized serum neutralizing antibody titers.[4] The duration of detectable circulating antibodies from exposure or vaccination may vary by individual host factors such as disease severity, vaccine dose regiment, age, or existing chronic infections.[8,9,14] In general neutralizing antibody titers have been shown to decline within 2-8 months, but protective titers against SARS-CoV-2 infection have yet to be determined.[10,19,21] But effective antibody responses cannot be characterized by durability alone.[4]

Even if a protective titer is identified, SARS-CoV-2 mutations on the RBD may render existing antibodies less protective.[4,16,19,17] Immune responses exert a selective pressure on the small repertoire of antibody accessible regions of the RBD.[5,16,29,30] The Spike protein structure utilized in both mRNA vaccines were designed to earlier lineages of SARS-CoV-2.[16,30] Booster vaccines may not improve antibody function, but they may continue to activate other effective immune components.[16] Nevertheless, antibody responses are not predictive of disease outcome.[25]

B cells

B cells are mainly thought to mediate the humoral response during primary SARS-CoV-2 infection.[2] When the adaptive immune response is activated, B cells migrate to the germinal center and differentiate into antibody secreting cells.[31,32] They may emerge as circulating plasma blasts (PBs) and produce antigen specific antibodies.[2,19,31,32] The terminally differentiated PBs are often shorted lived but long-lasting plasma cells have been identified in the bone marrow.[31,32] Alternatively, they may differentiate into memory B cells (MBCs) and expand after infection to produce more broadly reactive antibodies.[29,33] SARS-CoV-2 antigen persisting in the small intestine months after infection fuels somatic mutations in MBCs and result in antibody affinity maturation.[19,29,32] Levels of MBCs increase for 6 months following infection before

plateauing.[10,17,24,29] Studies of 2003 SARS-CoV infection suggest that MBCs can survive for several months to a year after infection but decline by 6 years post infection.[25]

During subsequent infection, circulating plasma cells may readily provide specific antibodies.[19] In the absence of circulating antibodies at reinfection, MBCs are responsible for the rapid reproduction of antigen specific antibodies.[2,9] Relatively early production of IgG in primary SARS-CoV-2 infection suggests that IgG MBCs may be cross-reactive with season coronaviruses (HCoVs).[33] IgG antibodies produced by cross-reactive MBCs correspond to the conserved S2 subunit but not the novel RBD region of SARS-CoV-2.[33] More in-depth investigations found that SARS-CoV-2 anti-S2 IgG antibodies cross-reacts most effectively with *Betacoronavirus* OC43, which is in the same genus as SARS-CoV-2.[4,3] Other studies have found serum antibodies of convalescent individuals that crossreact with the S2 domain of SARS-CoV and HCoVs but not the RBD of SARS-CoV-2.[24] Though MBCs provide promising cross-reactive capacity, more information is needed to determine how affinity maturation may improve their effectivity upon rapid restimulation.

T helper cells

CD4+ T cells offer a breadth of protection against SARS-COV-2 throughout the entire course of infection.[2,4,8,21] Though they have direct antiviral functions, CD4+ T cells are best known for their helper activity.[21] CD4+ T cells orchestrate the adaptive immune response by generating memory B cells, activating CD8+ T cells, and recruiting innate effector cells.[4,3,8,9] Nearly all infected individuals mount a CD4+ T cell response predominantly specific to the S, N, or M proteins.[3,4,8,2] The magnitude of T cell responses following natural infection is not impeded by disease severity or age.[8,34] Most CD4+ T cells in SARS-CoV-2 infection polarize into T-helper 1

(Th1) and follicular helper T (Tfh) cell subsets with relatively few polarized towards T-helper 2 (Th2) cell profiles.[3,4,21] Tfh cells are responsible for selection and maturation of antigen specific B cells in the germinal centers whereas Th1 cell response is responsible for recruiting effector cells such as CD8+ T cells or monocytes.[4,7,9] S-specific CD4+ T cells skew more towards circulating Tfh cell types, and N- or M-specific CD4+ T cells tend to polarize towards Th1.[21] SARS-CoV-2 specific CD4+ T cells predominantly produce IFNy, TNFa, and IL-2 cytokines, which are used as biomarkers to measure their response in laboratory settings.[2,21]

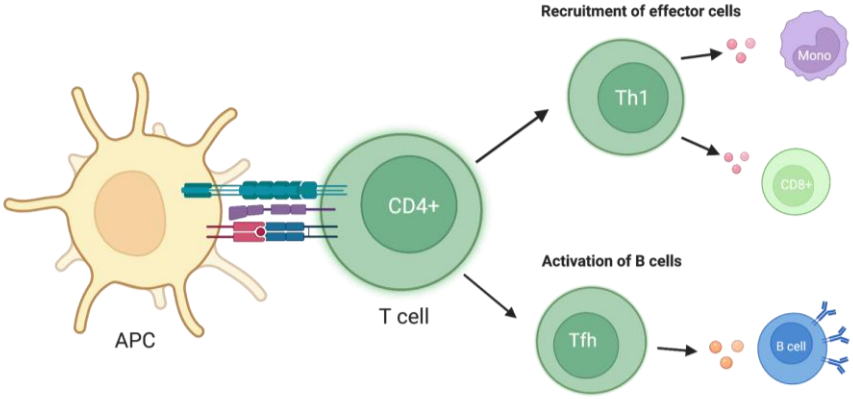

Figure 3. ***T helper activity*** Th1 responses recriut effort cells while Tfh response activate B cells and antibody production

T helper cell responses during secondary exposures rapidly produce antiviral cytokines and restimulate B cells antibody production.[2,3,7] S specific memory CD4+ T cells detected in infected, vaccinated, and unexposed individuals suggest their broad and cross-reactive potential.[6,35] The estimated 90% of the population that has been infected with seasonal coronaviruses (HCoVs) explain the cross reactive CD4+ T cell response detected in a majority of SARS-CoV-2 naïve

individuals.[2,4,7,17] CD4+ T cell responses generated from HCoVs provide a limited relief against SARS-CoV-2 but accelerate the immune response in infection and vaccination.[6,36] Consistent with known similarities across coronavirus S proteins, cross reactive T cells respond most abundantly to the S2 region and help quickly produce S2 specific antibodies (source). The novel RBD mutations may explain why T cell cross reactivity has limited effectivity during primary infection SARS-CoV-2 but will likely be improved following an exposure or vaccination.[6,37] Their broadly reactive potential has also been shown to protect against most S protein mutations.[16,17,23]

Promisingly, CD4+ T cells responses to 2003 SARS-CoV have been detected as far as 11 years after infection.[2] Early assessment of SARS-CoV-2 specific CD4+ T cell durability in infected individuals identified an initial decline followed by a stable plateau lasting at least 8 months in 92% of the cohort.[10] Though SARS-CoV-2 emerged only a few years ago, several studies have found long lasting potent T cell responses and predict that they will persist for years.[4,7,19,21]

Cytotoxic T cells

CD8+ cytotoxic T cells provide a direct antiviral response associated with less severe disease.[9] Their responses are mediated through that activity of Th1 cells and, like CD4+ T cells, they are broadly reactive to multiple SARS-CoV-2 proteins.[23,34] CD8+ T cells are similarly not susceptible to broad escape by most SARS-CoV-2 mutations and retain long-lasting potency.[10] CD8+ T cell responses may be measured by IFN-γ and CD107a production[21]. Most exposures result in a potent CD8+ T cell, yet many studies have not been adequately designed to accurately measure their responses *in vitro.*[4] When restimulated with 15-mer Spike peptides, CD8+ T cells are unable to efficiently recognize these unprocessed peptides in the context of MHC-class I

without the aid of professional antigen presenting cells, while these longer peptides can be presented directly on target cells in the context of MHC-class II, allowing for more efficient recognition by CD4+ T cells in the absence of an antigen presenting cell.[10] Therefore, these suboptimal detection methods may undermine the potency of CD8+ T cell responses and have led other studies to conclude that adaptive T cell responses to SARS-CoV-2 more skewed toward responsive CD4+ T cells.[3,21] Though CD8+ T cell detection methods may be improved, they are generally thought to have consistent and potent responses following infection and vaccination that can be recalled upon secondary exposure.

3.0 Summary

Adaptive Immune Response to SARS-CoV-2

The current assessment methods used to determine correlates of immunity to SARS-CoV-2 need improvements. Serum IgG neutralizing antibody titers are primarily used to measure protection against infection. Though this method is easiest to use in a large population, it does not accurately reflect the humoral response in the respiratory mucosa during exposures.[3,24,26] The dimeric form of IgA found in mucosal surfaces has a higher neutralizing capacity against the SARS-CoV-2 than the monomeric form found in serum.[27] Following a natural infection, IgA neutralizing antibodies in the mucosa are more concentrated and durable than in the blood.[26] Antibody measurements of saliva or nasal swabs may offer more accurate assessments of immune protection.

Variable assessments of SARS-CoV-2 neutralizing antibody responses thus far have not given us a clear picture of antibody effectiveness or durability. Antibody titers from infection and vaccination dramatically decline between 2 and 8 months after exposure.[10] Furthermore, antibody neutralizing potency is reduced against many circulating variants.[14] Thus, it is difficult to determine what antibody titers effectively protect against infection.

Circulating memory B cells may provide more effective antibody responses during subsequent exposures. In the 6 months following an infection, MBCs multiply and generate high affinity antibodies through somatic mutation.[31,32] Yet, little is known about their long-term durability.[10] Further characterizations of MBC activation during subsequent exposures are needed.

T cell responses following SARS-CoV-2 exposures are robust and durable.[21,37] Nearly all individuals exposed to SARS-CoV-2 develop T cell responses to Spike, Nucleocapsid, or

Membrane proteins.[2,4] T cells are responsible for activation of B cells and antibody secretion upon exposure and promoting effector functions during infection.[3,21] T cell responses are directly associated with better disease outcomes; however, their roles are not associated with prevention of infection establishment.[8] Broadly reactive T cells have not shown susceptibility to SARS-CoV-2 mutations.[17,35] T cell responses are detected months to years following exposure to coronaviruses. Not enough time has passed since the emergence of SARS-CoV-2 to evaluate their long-term durability.

4.0 Discussion

Correlates of Immunity

Considering the responsiveness and functionality of all these immune responses to subsequent SARS-CoV-2 infections, we can better characterize correlates of immunity. I propose that there are two distinct correlates to consider: effective immunity and supportive immunity. Effector correlates respond to protection against establishment of infection and prevention of further transmission. Supporter correlates respond to protection against symptomatic infection. This distinction is important for controlling the spread of SARS-CoV-2 in the population. High affinity neutralizing antibodies in the respiratory mucosa are more likely going to serve as an effector correlate of immunity by preventing viral entry into the cells where the pathogen is first deposited. Circulating neutralizing antibodies in the blood may be better associated with prevention of viral dissemination. Thus, I hypothesize that mucosal neutralizing antibody titers may serve as an effector correlate of immunity while serum neutralizing antibody titers are more of a supporter correlate of immunity. Further investigation is needed to understand this relationship. Additionally, I propose that memory B cells have potential to serve as an effector correlate of immunity due to their ability to rapidly produce high affinity antibodies against new SARS-CoV-2 mutations.[29,32,43] Further investigation of memory B cell kinetics after exposure is needed to understand their protective role. Lastly, I suggest that robust T cell responses serve as an effector correlate of immunity and will prevent against severe disease during subsequent infections. These distinctions may be clarified by improved understanding of asymptomatic to mild infections. Future research ought to investigate the distinct role of antibodies, B cell, and T cell subtypes on SARS-CoV-2 infectivity and transmissibility.

Vaccines

The two Covid-19 mRNA vaccines currently utilized in the United States were developed on the basis that robust anti-S antibody responses prevent infection.[38] These vaccines have shown to induce a strong humoral and cell-mediated immune response to the SARS-CoV-2 Spike protein.[38,39,41] Early evaluations of these vaccines assessed efficacy against symptomatic infection.[38] Based on the evidence presented in this paper, I hypothesize that Covid-19 vaccines do prevent symptomatic and severe infection, however, they may not effectively prevent transmissible asymptomatic infection. I suggest that this may be a result of the type and specificity of the antibody response. The S protein sequence encoded by the mRNA vaccine was derived from earlier lineages of SARS-CoV-2.[30] The most common mutations identified in circulating SARS-CoV-2 variants appear in the RBD amino acids targeted by human antibodies.[30,40] The mRNA vaccine induced antibody responses will likely become less effective as their similarities drive immune escape.[40] Additionally, since the intramuscular administration of this vaccine does not appear to produce a strong mucosal response, it may not be as protective at the initial site of infection.[18] Thus, the vaccines likely provide suboptimal protection against infection establishment, especially against new variant. Recognizing that the vaccine provides protection against disease severity but not infection should shift the basis of waning immunity models from neutralizing antibody titer to T cell responses.

Detecting a prior SARS-CoV-2 infection is critical for many efficacy-based study designs. The standard practice is to measure anti-N antibodies in serum. Because not all infections, especially asymptomatic or mild ones, result in a lasting detectable antibody response, this is not a reliable way to verify infection. Similarly, this method will not accurately detect infections that

occurred more than 2 to 8 months prior to sampling. Better results may be derived from detecting N specific T cell responses by ELISpot assay.

The information discussed in this paper may also inform future vaccine development. Vaccines designed to protect against symptomatic disease should stimulate a broad T cell response. Including more target proteins, such as the nucleocapsid or membrane proteins, may improve the elicited immune response and limit the virus's possible escape mutations. Vaccines designed to protect against infection establishment will need to produce a potent neutralizing antibody response at the site of initial infection. Intranasal vaccines should be prioritized due to their ability to stimulate a mucosal immune response. Research ought to consider memory B cell affinity maturation mechanisms as a potential target for providing a more sustainable antibody response.

5.0 Public Health Significance

The Covid-19 pandemic will not be controlled until immunity is widespread. Globally, vaccine distribution has been inequitable. In low-income countries, 16% of people have received at least one dose, compared to 72% in high income countries.[43] In the Unites States, boosters are now recommended 5 months after a primary series— even if you've recently had a breakthrough infection.[44] This recommendation comes from models of waning immunity based on neutralizing antibody titers. More accurate characterization of immune protection is necessary to optimize the global vaccine strategy. Additionally, a better understanding of how these individual immune dynamics influence disease burden and transmission will improve the larger public health strategy against the Covid-19 pandemic.

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