CELLULAR IMMUNE RESPONSES TO ENDEMIC CORONAVIRUSES, SARS-COV-2 AND COVID-19 mRNA VACCINATIONS

by
Bezawit Abi Woldemeskel

A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
May 2022

Abstract

Since its emergence in early 2019, the COVID-19 pandemic has caused millions of infections and fatalities globally. Recent evidence show that bats are reservoirs for numerous novel coronaviruses with zoonotic potential. Thus, it is important to understand and to characterize immune responses to human coronaviruses following infection and COVID-19 vaccinations.

Our adaptive immune system, comprised of B and T cells, is important for controlling and clearing viral infections. T cells have been shown to be important for controlling SARS-CoV and MERS-CoV infections. However, T cell responses to endemic coronaviruses have not been characterized. Furthermore, some studies have shown that a significant portion of COVID-19 unexposed individuals have pre-existing T cell response to SARS-CoV-2. However, the source of these T cells, and whether SARS-CoV-2 pre-existing T cells cross-react with endemic coronaviruses was unknown. Furthermore, the ability of T cells from COVID-19 mRNA vaccinated individuals to recognize peptides from bat coronaviruses that may have the potential of causing future pandemics was unknown.

Our data show that most healthy donors have robust T cell responses to three common cold coronaviruses tested. Furthermore, we show that current vaccine strategies enhance T cell responses to the endemic coronavirus HCoV-NL63. Additionally, we identified a SARS-CoV-2 spike protein epitope (S815-827) that is conserved in divergent coronaviruses including SARS-CoV, MERS-CoV, and multiple bat coronaviruses. Our results show that this conserved epitope is recognized by 42% of vaccinated participants who received COVID-19 mRNA vaccines. Using T cell expansion and T cell receptor sequencing assays, we also show that S815-827-reactive CD4+ T cells cross recognize

diverse coronaviruses. Moreover, we characterize targeted peptides following COVID-19 vaccinations, and show that vaccine-elicited T cells can cross-recognize SARS-CoV-2 variants of concern.

Our results suggest that current mRNA vaccines elicit T cell responses that can cross-recognize endemic coronaviruses and bat coronaviruses. Furthermore, our data provide important insights that inform the development of T cell-based pan-coronavirus vaccine strategies that can protect against future pandemics.

Primary Reader and Advisor: Joel Blankson MD, PhD

Secondary Reader: Andrea Cox MD, PhD

Acknowledgments

I would first like to express my deepest gratitude to my mentor Dr. Joel Blankson, for being a caring and patient mentor and for his constant excitement and enthusiasm for my work. Thank you for taking the time to give me immediate feedback, and for prioritizing my development and success in graduate school. I would also like to thank my lab mate Caroline Garliss for her friendship, her expertise and training, and for her work supporting my projects and managing the lab. I would like to thank Dr. Abena Kwaa for being a kind mentor throughout graduate school, for giving thoughtful advice during difficult times, and for sharing her knowledge and expertise to onboard and train me into the Blankson lab.

I would like to thank Dr. Rebecca Veenhuis for being available to answer my questions thoughtfully, thoroughly, and patiently. My deepest gratitude also goes to Dr. Kellie Smith and Arbor Dykema for being critical collaborators that ensured the success of our work. Thank you also to my thesis committee members: Dr. Andrea Cox, Dr. Stewart Ray, and Dr. Robert Siliciano for their time and feedback on my projects. I would also like to extend my gratitude to Dr. Laura Wood, Dr. Peter Espenshade and Dr. Damani Pigott for being non-scientific mentors to me and for their advice early in my graduate career.

My deepest appreciation, gratitude and love goes to my family for being a constant source of support and encouragement. To my parents, Abi Woldemeskel Bayou and Sehin Sahle Negatu, thank you for prioritizing my education. Thank you for your advice and guidance, for the love and laughter, and for always believing in my potential. A special thank you to my father who had a special love for the academic path

and to my mother for setting an impeccable example of emotionally resilience and strength. I would like to also thank my sisters, Selam and Betty Woldemeskel, for their constant friendship and support, and for setting an example of grace and ambition. Thank you to my aunt and uncle, Hana and Tesfaye, for providing me with a comfortable home in the US, and for making Holiday celebrations memorable and enjoyable.

I would like to thank my oldest and longest friend Bilal Alaji for being an exceptionally kind friend, confidant, and source of support. To my DC friends, and especially to Mignote Haile, thank you for providing a well needed break from grad school and Baltimore, and for being a fun dance partner. A special thank you to my Hopkins friends and especially to Kamsi Odinammadu for the study sessions, fun conversations, and long dance parties. Thank you to Phillip Scott for the fun and memorable conversations. And a special thank you to the Biomedical Scholars Association and especially to former president Sara Haile for providing me with a supportive community and sense of purpose when I needed it most.

Finally, I would like to thank Mount Holyoke College, for providing me with the financial support I needed to pursue a higher education in the US, without which I wouldn't be here today. And thank you to the CMM program for providing me with the opportunity to pursue my graduate education at Hopkins, and for striving towards creating a diverse student body.

Dedication

This work is dedicated to my parents Abi Woldemeskel Bayou and Sehin Sahle Negatu for their endless love, support, and sacrifice.

Table of Contents

Abstract ..... *ii*

Acknowledgments ..... *iv*

Dedication ..... *vi*

List of Tables ..... *x*

List of Figures ..... *xi*

1 Chapter 1: Introduction *1*

1.1 Introduction to coronaviruses **1**

1.2 Introduction to the adaptive immune system **4**

1.3 Adaptive immune responses to coronaviruses **6**

1.3.1 Humoral Immunity to COVID-19 infections 7

1.3.2 Cellular Immunity to COVID-19 infections 8

1.4 T cell cross-reactivity: Definition, Mechanism and Function **13**

1.4.1 Pre-existing T cell responses to SARS-CoV-2 18

1.4.2 Cross-reactive T cell responses in COVID-19 Convalescent Patients and COVID-19 mRNA vaccine recipients 19

1.4.3 Cross-reactive T cell mediated protection in the context of SARS-CoV-2 21

1.4.4 Cross-reactive antibody responses in the context of SARS-CoV-2 23

1.5 Objectives **23**

1.6 References **24**

2 Chapter 2: Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2 *44*

2.1 Abstract **44**

2.2 Introduction **45**

2.3 Methods **47**

2.4 Results **51**

2.5 Discussion **64**

2.6 References **68**

3 Chapter 3: SARS-CoV-2 mRNA Vaccines induce broad CD4+ T cell responses recognize HCoV-NL63 and the variants of concern *72*

3.1 Abstract **72**

3.2 Introduction **73**

3.3 Methods **74**

3.4 Results and Discussion **78**

3.5 References **95**

4 Chapter 4: CD4+ T-cells from COVID-19 mRNA vaccine recipients recognize a conserved epitope present in diverse coronaviruses ***98***

4.1 Abstract **98**

4.2 Introduction **99**

4.3 Methods **101**

4.4 Results **105**

4.5 Discussion **125**

4.6 References **128**

5 Chapter 5: mRNA vaccine-elicited SARS-CoV-2-specific T cells persist at 6 months and recognize the delta variant ***133***

5.1 Abstract **133**

5.2 Introduction **133**

5.3 Methods **134**

5.4 Results **135**

5.5 Discussion **139**

5.6 References **141**

6 Chapter 6: COVID-19 mRNA Vaccine-induced T cells recognize the Omicron variant ***142***

6.1 Abstract **142**

6.2 Introduction **143**

6.3 Methods **143**

6.4 Results **144**

6.5 Discussion **147**

6.6 References **148**

7 Chapter 7: SARS-CoV-2-specific immune responses in boosted vaccine recipients with breakthrough infections during the Omicron variant surge ***149***

7.1 Abstract **150**

7.2 Introduction **150**

7.3 Methods **152**

7.4 Results **158**

7.5 References **172**

8 Chapter 8: The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living With Human Immunodeficiency Virus (HIV) ***175***

8.1 Introduction **175**

8.2 Methods **176**
8.3 Results **177**
8.4 Discussion **179**
8.5 References **181**
9 Chapter 9: Decay of COVID-19 mRNA vaccine-induced immunity in people living with HIV 182
9.1 Introduction **182**
9.2 Methods **183**
9.3 Results and Discussion **183**
9.4 References **187**
10 Chapter 10: Concluding Remarks ***190***
10.1 Summary **191**
10.2 Future Directions **192**
10.3 References **194**
11 Curriculum Vitae ***199***

List of Tables

Table 1.1: List of human coronaviruses 4

Table 2.1: HCoV-NL63 T cell-targeted peptides detected by ELISpot assay and HLA-binding predictions 63

Table 3.1: Peptides targeted by vaccine recipients' CD4+ T cells 90

Table 3.2: Complete list of peptides targeted by vaccine recipients' CD4+ T cells 91

Table 4.1: List of coronaviruses peptides and sequences used in this study.....121

Table 4.2: S815-827 is identical in sequence between SARS-CoV-2 and SARS-related coronaviruses in the subgenus sarbecovirus and genus betacoronavirus listed. 121

Table 4.3: Class II HLA alleles of vaccinated individuals who responded to $S_{815-827}$. ...122

Table 7.1: List of vaccine recipients who experienced breakthrough infections after booster shots (breakthrough VRs) 157

List of Figures

Figure 1.1: Illustration of cross-reactive T cells.. 17

Figure 2.1: IFN-γ responses to viral peptide pools from HDs and CCPs.. 53

Figure 2.2: INF-γ responses to CEF and unfractioned PBMCs and CD8 Depleted PBMCs. 54

Figure 2.3: Breadth of T cell responses to HCoV-NL63 protein.. 56

Figure 2.4: Expansion of antigen-specific CD4+ T cell responses.. 59

Figure 2.5: Cross-recognition of HCoV-NL63 and SARS-CoV-2 S protein peptide pools in HD9 and CCP2.. 60

Figure 2.6: Expansion of antigen-specific memory CD4+ and CD8+ T cell responses from a COVID-19 convalescent patient (CCP3).. 61

Figure 2.7: Expansion of antigen-specific memory CD8+ T cell responses.. 62

Figure 3.1: T cell IFN-γ responses to SARS-CoV-2 and CCCs.. 80

Figure 3.2: T cell IFN-γ responses to SARS-CoV-2 and CCCs (presented differently)……81

Figure 3.3: CD4+ T cell responses to SARS-CoV-2 and CCCs. 84

Figure 3.4:HCoV-NL63 SpecificCD4+ T cell responses to re-stimulation by SARS-CoV-2 and CCC peptide pools.. 85

Figure 3.5: Breadth of T cell responses to SARS-CoV-2 spike protein and responses to spike variants.. 88

Figure 4.1: Some individuals vaccinated with COVID-19 mRNA vaccines have CD4+ T cells that recognize the conserved SARS-CoV-2 epitope $S_{815-827}$ and homologous peptides from diverse coronaviruses.....108

Figure 4.2: $S_{815-827}$ reactive T cells are not detected in pre-vaccination samples, and have similar functional avidity to T cells reactive to homologous peptides.. 110

Figure 4.3: $S_{815-827}$ specific CD4+ T cells respond to re-stimulation with homologous peptides from diverse coronaviruses.....112

Figure 4.4: CD4+ T cell clonotypes that cross-recognize both $S_{815-827}$ and homologous peptides from diverse coronaviruses are present in vaccinated donors 115

Figure 4.5: Cross-reactive CD4+ T cell clonotypes that react to diverse coronaviruses are found in vaccinated donors. 117

Figure 4.6: Cross-reactive CD4+ T cell clonotypes that react to diverse coronaviruses (excluding $S_{815-827}$) are found in vaccinated donors. 119

Figure 5.1: T cell responses of COVID-19 mRNA vaccinated individuals at different timepoints.. 138

Figure 6.1: Antibody and T cell responses to vaccine strain and Omicron variant spike proteins.. 146
Figure 7.1:Design of observational study of breakthrough boosted vaccine recipients. 159
Figure 7.2:Characterization of antibody levels in breakthrough boosted vaccine recipients.. 162
Figure 7.3:Correlation of ACE2 inhibiting antibodies to days since symptom onset.. .. 164
Figure 7.4:Characterization of SARS-CoV-2 specific T cells in breakthrough boosted vaccine recipients.. 166
Figure 7.5:Characterization of immune responses following breakthrough infections in breakthrough VRs who only received mRNA vaccinations.. 167
Figure 7.6: Characterization of longitudinal SARS-CoV-2-specific antibody and T cell responses in breakthrough boosted vaccine recipients.. 170
Figure 7.7:Antibody and T cell responses to spike and nucleocapsid proteins. 171
Figure 8.1: Characterization of immune responses in People living with HIV (PLWH) compared to Healthy Donors (HDs).. 178
Figure 8.2:CD4 T cell responses in People living with HIV (PLWH) compared to Healthy Donors (HDs.. 179
Figure 9.1: Antibody and T cell responses in PLWH and healthy controls at peak and six-months following COVID-19 mRNA vaccinations.. 186

1 Chapter 1: Introduction

1.1 Introduction to coronaviruses

Coronaviruses are a large family of viruses with single-stranded, positive sense RNA genomes of almost 30kb in size, the largest known genome for any RNA virus [1-3]. They are named after the latin word “corona” which means “crown” to reflect their appearance while viewed under an electron microscope, which results from the protrusion of spike proteins on their surface [4]. Coronaviruses were first discovered in the mid 1960s, when a common cold coronavirus was isolated from a patient experiencing upper respiratory symptoms [1]. Since then, six other coronaviruses that infect humans have been discovered [5-12] (Table 1.1).

In 2002-2003, the severe acute respiratory syndrome (SARS) emerged in China [10]. It is thought to have bat origins but transmitted through civet cats as an intermediate host [1,2] . It had a 10% fatality rate, and infected about 8000 people [1,2]. In 2012, the MERS pandemic emerged in the middle east [11], with an even higher fatality rate of 35% [1,2]. MERS had dromedary camels as an intermediary host but is also thought to have evolutionary origins in bats [1-3]. Most recently, the COVID-19 pandemic emerged in Wuhan China [12]. COVID-19 is caused by SARS-CoV-2 and is a highly transmissible relative to previous coronaviruses but has a lower fatality rate of 2-4% percent [3]. Despite this, it has caused a tremendous toll on the global population, causing hundreds of millions of infections and millions of fatalities [13]. In addition to pandemic causing coronaviruses, there are also four endemic coronaviruses that cause self-limiting mild respiratory symptoms: HCoV-NL63, HCoV-OC43, HCoV-229E and HCoV-HKU1 [1]. Most

human coronaviruses are thought to have ancestral origins from bats, and bat coronaviruses related to endemic and pandemic causing coronaviruses have been isolated [1,2,14-17] .

Coronaviruses belong to the family Coronaviridae and subfamily Orthocoronavirinae, which is divided into the genera: alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus [18,19]. Human coronaviruses are classified into the genera alphacoronavirus (NL63, 229E) and beta coronaviruses (OC43, HKU1, SARS, MERS and SARS-CoV-2) [1]. All coronaviruses share similar organization of their genomes including 16 nonstructural proteins encoded at the 5'end followed four structural proteins: Spike (S), envelope (E), membrane (M) and nucleocapsid (N) [20]. The spike protein, which is a homotrimer, dictates host tropism and is used for receptor recognition, binding, and entry [3,20]. Spike includes two subunits, S1 and S2 [3,20]. The S1 contains the receptor binding domain, whereas the S2 domain contains the fusion peptide, and needs to be proteolytically cleaved at the S2' site, upstream of the fusion peptide in order to activate the protein for membrane fusion [20].

The S2 domain of the spike is more conserved across coronaviruses, whereas the S1 domain and the receptor binding domain are more divergent [1,3]. As a result, most human coronaviruses have different host receptors [21-28] (listed in table 1.1). SARS and SARS-CoV-2 are more closely related, with about 80% sequence similarity and belong to the same species [3,18,19]. On the other hand, SARS-CoV-2 has less than 30% sequence similarity with the other human coronaviruses [3] .

Since its emergence in 2019, the novel coronavirus SARS-CoV-2 has also evolved, likely because of the large number of individuals that continue to be infected

globally, and potentially prolonged infections in immunocompromised individuals [29]. Variants of Concern (VOCs) are defined by the WHO as a SARS-CoV-2 virus with mutations that either increase transmission or virulence and decrease the impact of vaccines and therapies. Thus far, there have been five VOCs: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2) and most recently Omicron (B.1.1.529) [30]. These variants have multiple mutations, primarily in the spike protein, that have resulted in increased infectivity and immune evasion [29] (discussed in more detail in following chapters).

Coronavirus pandemics are an imminent threat to the human race [31]. Several coronaviruses with close relationship to MERS-CoV, SARS-CoV and SARS-CoV-2 have been isolated from bats worldwide [15,32-37]. Furthermore, mutations in SARS-CoV-2 and the rise of variants of concern that are more transmissible and evade immune responses continue to cause global concern [38,39]. Thus, the study of coronavirus biology, and immune responses to these viruses is important for the continued development of therapies and vaccines. In this thesis, I characterize T cell immune responses to SARS-CoV-2 and endemic coronaviruses following natural infections and COVID-19 vaccinations. We begin with a brief introduction of the adaptive immune system.

Table 1.1: List of human coronaviruses

Virus	Discovery	Prevalence	Origin	Symptoms	Fatality	Receptor	Sequence Similarity to SARS-CoV-2
HCoV-229E	1966	epidemic	likely bat	mild upper respiratory diseases	-	Aminopeptidase N	<30%
HCoV-OC43	1967	epidemic	likely rodents - bovine intermediary host	mild upper respiratory diseases	-	9-O-acetyl-sialic acid	<30%
HCoV-NL63	2004	epidemic	likely bat	mild upper respiratory diseases	-	ACE2	<30%
HCoV-HKU1	2005	epidemic	likely rodents	mild upper respiratory diseases	-	9-O-acetyl-sialic acid	<30%
SARS-CoV	2002	pandemic	likely horseshoe bats - civet cat intermediary	severe respiratory syndrome	10%	ACE2	~80%
MERS-CoV	2012	pandemic	likely bat - dromedary camels intermediary	severe respiratory syndrome	36%	DDP4 (CD26)	<30%
SARS-CoV-2	2019	pandemic	likely horseshoe bats -likely direct transmission	severe respiratory syndrome	3-4%	ACE2	100%

1.2 Introduction to the adaptive immune system

The immune system is broadly divided into the innate and adaptive immune response, with each arm having distinct but synergistic functions, and comprised of unique cell types. Adaptive immunity, which is composed of B and T cells, is defined by two distinct features: specificity and memory. Specificity, explained by the clonal selection theory, refers to the ability of adaptive immune cells to recognize specific antigens, through highly variable receptors made possible by somatic gene rearrangements (VDJ recombination). Each lymphocyte expresses a unique receptor against a specific antigen on its cell surface which is used for antigen recognition and response. A population of lymphocytes thus expresses billions of receptors that are highly diverse and allow for the specific recognition of a wide array of infectious agents. It is estimated that naïve B and T cell lymphocyte repertoires consists of 10^9 different antigen-specific receptors [40].

Once antigen encounter occurs, adaptive immunity is slow to mount and takes days to weeks to peak, since specific antigen recognizing cells must proliferate and differentiate into effector cells. Memory B and T cells are also established that persist for years and are able to respond with faster kinetics and greater magnitude upon re-exposure. This immunological memory of the adaptive immune system is thus the basis of vaccine design [40.]

T cells and B cells express distinct receptors on their cell surface and have different functions in the immune system. B cells express a class of immunoglobulin (Ig) molecules on their cell surface, which is used for antigen recognition. Upon activation, effector B cells differentiate into plasma cells that secrete antibodies with the same specificity as the original activated B cell. Furthermore, B cells undergo complex processes, such as affinity maturation and class-switching, by which they enhance the specificity of antibody recognition and effector function of antibodies. Antibodies recognize proteins and glycoproteins in their native confirmation, and have important functions such as neutralization of infectivity, and antibody-mediated functions such as antibody mediated phagocytosis [40].

T cells are divided into CD4+ and CD8+ T cells, with each having important effector functions in the immune system. CD8+ T cells (also known as cytotoxic T cells/lymphocytes or CTLs) are important for the direct killing of infected cells and for viral clearance. CD4+ T cells have varied effector functions, including helper T cell functions important for the generation of proper antibody and CD8+ T cell functions. In contrast to B cells, T cells express T cell receptors (TCRs) on their cell surface and recognize peptides that are presented in the context of major histocompatibility (MHC)

molecules. MHC molecules are encoded by the HLA locus, which is highly polymorphic. Each individual expresses three different MHC class I molecules (HLA-A, HLA-B, HLA-C) that present endogenous peptides, and three different MHCII molecules (HLA-DR, HLA-DP, HLA-DQ) which present exogenous peptides. CD8+ T cells recognize peptides 8-10 amino acids in length that are presented in the context of MHC class I molecules and are found on the surface of almost all cell types. In contrast, CD4+ T cells recognize slightly longer peptides of 12-15 amino acids that are presented on MHC class II molecules on the surface of antigen presenting cells (macrophages, B cells and dendritic cells). [40] Unlike B cells, T cell receptors do not have a soluble form of their TCR and do not undergo further affinity maturation after antigen recognition.

In this thesis work, I characterize the magnitude and breadth of T cell responses to endemic common cold coronaviruses and SARS-CoV-2 following infections and vaccinations using various tools. With collaborators, we also analyze the magnitude of binding and neutralizing antibodies responses. Collectively, this work contributes to our understanding of immune responses to the novel coronavirus SARS-CoV-2 that emerged in 2019 and informs the development of future vaccine strategies that harness the immune response. In the following sections, I summarize the current, evolving literature on adaptive immune responses to SARS-CoV-2 with a specific focus on T cell responses. Further, I put into context the work that I have done during my thesis research relative to the current literature and will reference manuscripts that have been published since my thesis work was published.

1.3 Adaptive immune responses to coronaviruses

1.3.1 Humoral Immunity to COVID-19 infections

Following SARS-CoV-2 infections, most individuals (>90%) develop antibodies to the virus within two weeks [41] [42]. Of interest for preventing infection is the development of strong neutralizing antibodies. COVID-19 infected individuals produce robust neutralizing antibodies against SARS-CoV-2. Most neutralizing antibodies target the receptor binding domain (RBD) and N terminal domain of S1whereas a small portion target the S2 domain of spike near the fusion peptide [41] [42]. It has been shown that the immune system can generate effective neutralizing antibodies with little affinity maturation [41,42], which suggests that the spike protein is highly immunogenic and production of antibodies that can neutralize the virus is relatively easy for the immune system.

After initial infection, a decline in antibody levels is expected. In the case of SARS-CoV-2, antibody levels decline dramatically within a matter of months[41]. However, memory B cells, which upon re-infection should rapidly mobilize to generate antibodies, are maintained up to 8 months following infection [43,44] . Furthermore, memory B cell responses seem to increase a few months following infection, suggesting an evolution in the humoral immune response [43,44]. Natural SARS-CoV-2 infections are shown to lower the risk of re-infections up to 7 months following infection [45]. However, the decline in antibody levels increase susceptibility to re-infections, suggesting that long-term sterilizing immunity resulting from natural infections might be unlikely, especially given the rise of variants of concern that can evade neutralizing antibodies [39]. Re-infections are also observed with endemic coronaviruses within six months [46] and reinfection with viral shedding was reported a year after initial infection [47].

Antibody levels to MERS and SARS are broadly similar to responses seen for SARS-CoV-2 infections, with 80-100% of patients having detectable antibody levels about two weeks of symptom onset [48]. A dramatic decline in antibody levels was also observed[49], and memory B cells were not detected after 6 years, [50] although these were done with ELISpot assay, which might have lower sensitivity [42,48] . The decline in coronavirus specific antibody levels contrasts with the longevity of T cell responses observed in endemic and pandemic causing coronaviruses, which are described in later sections.

1.3.2 Cellular Immunity to COVID-19 infections

T cell responses in COVID-19 convalescent patients have been extensively studied, and the function, kinetics, magnitude, breadth of epitope recognition, phenotype, longevity, and pre-existing and cross-reactive T cell responses have been described in the literature. In the following sections, I will briefly summarize the cellular immune response following COVID-19 infection and vaccination.

1.3.2.1 T cell mediated protection against coronavirus infections

Simplistically, neutralizing antibodies are important for preventing infection, whereas T cells are necessary for rapid clearance of viral infections once established [42]. T cells are comprised of CD8+ T cells, which have cytotoxic functions and kill infected cells, and CD4+ T cells, which have effector and helper functions such as secretion of cytokines and facilitating the development of durable antibody responses. CD4+ and CD8+ T cells have been associated with a mild disease course following infection [51,52]. Early, activated CD8+ T cells (HLA-DR+CD38+) are seen in higher numbers in mild

disease [53], and reduced CD8+ T cell frequency have been associated with severe disease [54]. Furthermore, severe COVID-19 has been associated with lymphopenia and delayed T cell responses [55,56], suggesting that T cells might be important for COVID-19 disease control.

Further evidence for the role of T cells in clearing COVID-19 infections comes from reports that show that some exposed individuals have T cell responses but do not seroconvert, suggesting that T cells might have cleared infections without sufficient exposure to elicit antibodies [52,57-59]. Moreover, individuals with deficiencies in humoral immunity, such as those with common variable immunodeficiency, have been shown to clear COVID-19 infection, suggesting that they relied on T cell responses for viral clearance [60]. Patients with hematologic cancers, including those that were on anti-CD20 therapy, with robust CD8+ T cell responses have also been shown to have increased survival compared with those with low CD8+ T cell responses [61]. Thus, although individuals with B cell deficiencies are at higher risk of severe disease [62], viral clearance in these patient populations highlights the important role of T cells in clearing SARS-CoV-2 infections.

Studies looking at human COVID-19 infections tend to be observational and correlative, and COVID-19 disease severity is also affected by a complex interplay with the innate immune system and other risk factors such as age and co-morbidities. Thus, animal models have been useful in demonstrating the role of T cells in viral clearance. Evidence from mouse models of SARS and MERS demonstrate the role of CD4+ and CD8+ T cells in acute viral clearance. Zhao et al 2016 has shown that airway memory CD4+ T cells protect mice against SARS challenge via IFN-γ production, and that

depletion of these memory T cells prior to challenge abrogated protection [63]. Furthermore, adoptive transfer of in vitro generated T cells to SCID mice enhanced survival and reduced virus titers in the lung after SARS-CoV infections [64]. Additionally, CD8+ T cells were shown to protect against a lethal dose of SARS-CoV in the absence of B cells and CD4+ T cells [65]. Similarly, in MERS mouse models, mice lacking B cells were able to clear infections [66].

More recently, animal models of SARS-CoV-2 have reached similar conclusions. Israelow et al 2021 have shown that mice lacking B cells cleared SARS-CoV-2 infections, although at a slower rate, showing that T cells are sufficient to clear infections in the absence of B cells [67]. Furthermore, depletion of either CD4+ or CD8+ T cells led to viral persistence, with depletion of both T cell subsets leading to severe persistence, similar to mice that lack both B cells and T cells. CD4+ T cell depletion was also shown to reduce spike specific antibody responses, highlighting the importance of T cell help for generating antibody responses [67] . Vaccination eliciting T cell responses alone without neutralizing antibodies was also shown to protect from SARS-CoV-2 infections in a mouse model [68]. Depletion of CD8+ T cells in convalescent macaques was also shown to partially abrogate protection, highlighting the importance of T cells in clearance of SARS-CoV-2 infections [69].

1.3.2.2 T cell responses in COVID-19 Convalescent Patients

Following SARS-CoV-2 infections, most individuals develop robust CD4+ and CD8+ T cell responses, with peak responses seen two weeks post symptom onset [42,55]. Cohen et al has estimated that the magnitude of T cell responses following infections to

be 0.5% and 0.2% of the repertoire for CD4+ T cells and CD8+ T cells, respectively [43,55]. SARS-CoV-2 specific T cells have been shown to be polyfunctional, with CD4+ T cells having a Th1 phenotype and expressing IL2 and INF-y (and a Th2 phenotype has been associated with severe disease) during infection [42,55]. T follicular helper cells are also expressed in high numbers and have been shown to correlate with neutralizing antibody responses [70], whereas CD8+ T cells show an activated and cytotoxic phenotype [42,55] .

T cell responses, particularly to the spike protein, seem to be dominated by CD4+ T cells [42,55], and CD8+ T cells seem to preferentially target the nucleocapsid protein [43]. However, both CD4+ and CD8+ T cells target the entire proteome of SARS-CoV-2, including the structural proteins and non-structural proteins [42,55]. Furthermore, the magnitude of T cell responses correlates with the level of protein expression of each SARS-CoV-2 gene. [42,55]

SARS-CoV-2 specific T cells target many epitopes, and it is estimated that within each individual, CD4+ T cells target about 19 specific epitopes and CD8+ T cells target about 17 epitopes [55,71]. Collectively, several research groups so far have identified over 1500 CD4+ epitopes and 1000 CD8+ epitopes [55,71]. Epitopes that are recognized across many individuals and are immunodominant are also being uncovered. Recently, NP105-113 was shown to be an immunodominant epitope of CD8+ T cells and to correlate with protection against severe disease [72]. Furthermore, a CD4+ immunodominant epitope within the receptor binding domain (RBD) S346-365 was shown to be recognized by 94% of individuals [73]. A CD4+ T cell immunodominant epitope within the fusion domain

of SARS-CoV-2 was also described (S816-830), and shown to be an important cross-reactive epitope [74].

Unlike the dramatic decline seen in antibodies, T cell responses have greater longevity and durability [42-44,55]. It has been shown that T cells are stable 12 months following infection [75], but there is hope that they will be maintained for years based on experience from SARS-CoV, where SARS-CoV specific memory T cells were detected 17 years after infection [76]. The magnitude, breadth and longevity of T cell responses following COVID-19 infections is encouraging, especially in light of variants of concern (discussed below), and highlights the importance of T cell based COVID-19 vaccine development.

1.3.2.1 T cell responses in COVID-19 mRNA vaccinated individuals

Vaccines harness immunological memory by eliciting memory B and T cells capable of recall upon infections. Since 2021, several COVID-19 vaccines that use the ancestral SARS-CoV-2 strain for template design have been developed and administered worldwide [29]. The mRNA COVID-19 vaccines made by Pfizer (BNT162b2) and Moderna (mRNA-1273), which use a pre-fusion stabilized spike protein, induce robust immune responses, and show remarkable efficacy (>90%) against the ancestral SARS-CoV-2 [77,78].

The COVID-19 mRNA vaccines elicit robust binding antibodies and neutralizing antibodies against the receptor binding domain of the spike protein [79]. Moreover, mRNA vaccines elicit strong CD4+ T cell responses (that are primarily of the Th1 phenotype), INF-y secreting CD8+ T cells [79,80], and T follicular helper cells [80-82]. Similar to natural

infections, the magnitude of CD8+ T cells seem to be lower compared to CD4+ T cells [83], and CD8+ T cells may wane more rapidly [82].

Vaccine induced immunity, particularly neutralizing antibody responses, wane over time [84,85] and are accompanied by a decline in vaccine efficacy against symptomatic infection [83,86]. However, memory T cell responses seem to be relatively stable over time, similar to observations made after natural infection [80,82,85] and might explain continued protection against severe disease.

Furthermore, COVID-19 vaccines have reduced efficacy in certain patient populations and in older adults, necessitating additional doses [80]. In this thesis research, I describe studies that were done to characterize immune responses to COVID-19 mRNA vaccinations, specifically focused on vaccine-induced T cell peptides and cross-reactive epitopes (chapter 3-4), responses against variants of concern (chapter 5-6), and immune responses in people living with HIV (PLWH).

1.4 T cell cross-reactivity: Definition, Mechanism and Function

T cells recognize peptides via the T cell receptor (TCR), a heterodimer of alpha and beta chains, presented to them in the context of MHC molecules. The naïve T cell receptor repertoire is very large and is estimated to include 10^8 unique TCRs [40]. This is made possible by somatic gene rearrangements of variable (V) and joining (J) segments in alpha chain and variable (V), joining (J) and diversity (D) segments in beta chains, coupled with combinatorial diversity introduced when alpha and beta chains are paired. Peptide-MHC recognition by TCRs is mediated by hypervariable complimentary determining regions (CDRs). The CD3 region which spans the VJ and VDJ junctions is

thought to determine peptide recognition [87]. Experimentally, the CD3 region can thus be used to identify a unique TCR and its cognate T cell clones in sequencing-based assays (such as the viral functional expansion of specific T cells (ViraFEST) [88] developed by Dr. Kellie Smith and described in subsequent chapters of this thesis).

In the clonal selection theory, it is thought that a unique TCRs recognizes a unique MHC-peptide combination [40]. However, it has been shown that T cell receptor recognition can be degenerate [89-91], and T cell cross-reactivity has been reported in different disease contexts including more recently SARS-CoV-2 [42,55,92]. T cell cross reactivity is defined as the same T cell receptor recognizing more than one peptide-MHC combination (Figure 1.1). The exact molecular and structural mechanism for T cell antigen specificity and cross-reactivity remain to be defined, but possible mechanisms include flexibility and promiscuity in binding of TCR:MHC, MHC:peptide or TCR:peptide (via CD3 conformational plasticity) [90,93]. A likely cause of cross-reactive T cell responses is also sequence homology between peptides. It has been suggested that certain TCRs focus on two-four upward facing peptide residues for antigen recognition, which might allow binding promiscuity at the TCR interface recognizing the peptide and be more forgiving of certain peptide residue changes [90]. Regardless, the exact structural mechanisms that allow cross-reactivity have not been fully characterized, thus computational predictions remain a challenge, and it is not yet possible to predict TCRs recognizing a given antigen or vice versa [94].

Functionally, Don Mason and Andrew Sewell have argued that T cell cross-reactivity might be necessary for proper immune surveillance of pathogens by the adaptive immune system [90]. Their estimates suggest that the number of possible

pathogenic peptide:MHC complexes far out way the TCR alpha and beta chains, and suggest that cross-reactivity is needed for the immune system to be able to recognize and protect against a wide array of pathogens. Generous estimates have predicted that a single TCR may be able to recognize 10^4 - 10^6 different MHC-associated epitopes [91], however, the experimental and clinical observations seem to be much lower. This highlights that the frequency of T cell cross-reactivity is still not fully understood and needs to be defined. [94]

T cell cross-reactivity has been associated with both protective and determinantal clinical consequences. A protective consequence is shown by the phenomenon of heterologous immunity, where exposure to one pathogen can protect against a closely related pathogen. An example of this is immunity comes from cowpox protecting against smallpox [90,95]. Vaccination with BCG (Bacille Calmette Guerin) vaccine against Mycobacterium tuberculosis infections has also been shown to protect against leprosy caused by M. leprae [96]. Cross-reactive pre-existing T cell immunity has also shown to be protective against infection during the 2009 H1N1 pandemic [97,98]. Recently, it has been suggested that immunity for endemic common cold coronaviruses might protect against SARS-CoV-2. Sagar et al have shown that individuals with confirmed prior infections with endemic common cold coronaviruses had better outcomes following COVID-19 infections [99]. Higher cross-reactive immunity was also reported in individuals who had a known exposure to SARS-CoV-2 but remained PCR negative [100], suggesting a potential role in protection from infection (discussed in more detail the following section).

T cell cross-reactivity from unrelated and dissimilar pathogens such as T cells for human papillomavirus peptide recognizing a coronavirus, and a flu specific CD8 T cell recognizing an Epstein-bar epitope have also been described [90], although the mechanisms and functional consequences are not known. Similarly, for SARS-CoV-2, cross-reactive T cells for non-homologous regions of CCCs have been reported,[74] suggesting that other pathogen derived peptides might lead to cross-reactivity. Indeed, some studies have shown that BCG vaccines [101,102] might generate T cells that cross-react with SARS-CoV-2, further highlighting that cross-reactivity can arise from unrelated pathogens, although the extent and functional importance of this cross-reactive cells still needs to be defined.

A potential negative consequence of cross-reactive immunity is described by the phenomena of original antigenic sin. This phenomenon is perhaps the flip side of heterologous immunity, where exposure to an antigen lead can lead to a suboptimal response to a subsequent closely related antigen, as the immune system relies on memory cells from the first exposure instead of generating new responses [103]. This phenomenon has been described in the context of flu and dengue exposure[104]. In the context of T cells, it has also been hypothesized that the original antigen sin can lead to skewed T cell responses, so, for example, a Th2 dependent response instead of Th1 mediated responses [90].

A further consequence of cross-reactivity is related to autoimmunity and related pathogenesis [105]. It has been noted that certain viral infections might lead to autoimmunity, likely via cross-reactive memory T cell responses that can be self-reactive. This might be likely due to weakly self-reactive T cells that survive selection in

thymus. Following infections, it is possible that a virus-specific memory T cell might be stimulated by a self-peptide with an affinity much lower than the original pathogen derived peptide leading to autoimmune responses [90] . Therapeutically, cross-reactivity can also be an issue. It has been shown that a T cell-based treatment for melanoma caused autotoxicity due to cross-reactive T cells that recognized self-proteins expressed on healthy cardiac cells [94,106] .

However, the exact structural mechanism, function and frequency of cross-reactivity remains to be fully elucidated. These responses likely vary based on pathogenic context and might have important protective or harmful consequences.

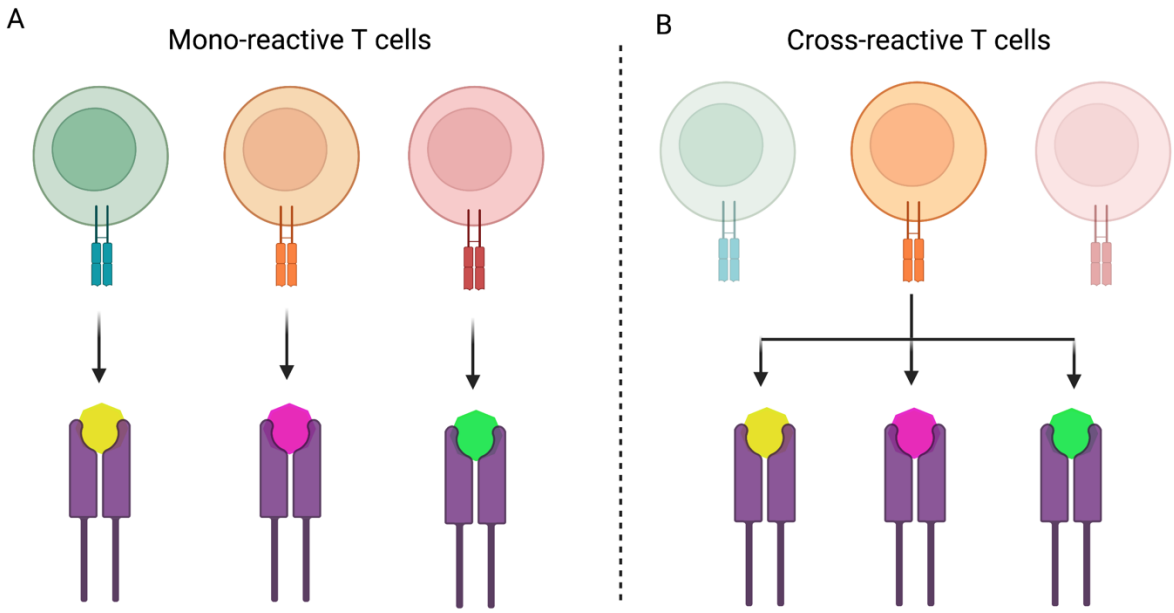

Figure 1.1: Illustration of cross-reactive T cells. Each T cell clone has a unique T cell receptor (TCR) resulting from somatic recombination during thymic development. In the clonal selection theory, it is suggested that one T cell clone recognizes only one peptide-MHC complex (A). However, it has been argued that T cells can also be cross-reactive. Cross-reactivity is defined by the ability of one T cell clone to recognize more

than one peptide-MHC complex (B). Cross-reactive T cells have been reported in infectious diseases such as the flu and more recently in COVID-19. Illustration done with biorender.com.

1.4.1 Pre-existing T cell responses to SARS-CoV-2

Pre-existing immunity to SARS-CoV-2 was reported in early 2020, when different groups observed SARS-CoV-2 reactive T cells in blood obtained prior to the COVID-19 pandemic. It was observed that 20-80% of individuals had pre-existing T cell responses [59,76,107-111]. Variation in frequency of pre-existing T cells is likely a result of differences in assays used (ELISpot vs activation induced marker (AIM+ assays)), and potentially differences in immune exposures in different geographies. T cell responses were observed for both structural proteins (spike and nucleocapsid) and non-structural proteins, but the spike region seems to be immunodominant with the more conserved C-terminal S2 domain of spike exhibiting more T cell reactivity [74]. Additionally, more robust responses were seen in CD4+ T cells relative to CD8+ T cells [92]. It was speculated that these cross-reactive T cells might be a result of T cells that are primed by the four endemic common cold coronaviruses (CCCs) [74,92]. Serology studies have shown that >90% of individuals have been exposed to CCCs [112,113], and that re-infections are frequent and seasonal [46,114]. Despite the prevalence of these viruses, and the discovery of the first endemic coronavirus in the 1960s however, characterization of cellular immunity to endemic coronaviruses in the literature was very limited.

Thus, it was important to understand T cell immune responses to common cold coronaviruses and identify targeted epitopes. In chapter 2 of this thesis (in a manuscript

published in 2020), I describe work that we did to characterize T cell responses to three common cold coronaviruses in a cohort of donors from Baltimore, MD [115]. We found that individuals have robust T cell responses to the spike protein of the three endemic coronaviruses that we tested, with more robust, higher frequency responses seen to HCoV-NL63. T cell responses were also broad and targeted the entire spike protein of HCoV-NL63 [115]. Recently, Yu et al did a longitudinal analysis of memory T cell responses to CCCs over a three-year timeframe [116]. Consistent with our results, they found that memory T cells to CCCs were robust and readily detectable using the AIM+ assay. They also show that T cell responses are sustained over time, and that sustained responses were unlikely a result of re-infections in their cohort [116].

1.4.2 Cross-reactive T cell responses in COVID-19 Convalescent Patients and COVID-19 mRNA vaccine recipients

In early 2020, it was speculated that the source of pre-existing responses was a result of cross-reactivity with CCCs which share some sequence homology with SARS-CoV-2 [92]. There is now strong evidence to support this hypothesis. In their recent study, Yu et al, found that robust T cell responses to CCCs correlated with pre-existing T cell responses to SARS-CoV-2 [116]. In our study, 1/21 healthy donors tested had a pre-existing T cell response, and many individuals with robust responses to CCCs did not have detectable pre-existing SARS-CoV-2 responses using the IFN-γ ELISpot assay [115]. This could likely be due to the reduced sensitivity of ELIspot compared to the AIM+ assay, and our inability to detect memory T cells of low frequency. However, in the donor where we found pre-existing T cell responses to SARS-CoV-2, we were able to show that CD4+ T cells were cross-reactive to HCoV-NL63 and SARS-CoV-2 by

generating T cell lines. Similarly, Mateus et al in a large cohort was able to demonstrate that pre-existing T cells in COVID-19 unexposed donors were partly a result of CCC cross-reactive T cells. In their assay, they were able to detect low frequency memory T cells by expanding cells for 14 days with peptide pools, before doing fluorospot assays to identify SARS-CoV-2 epitopes in unexposed donors. They subsequently generated T cell lines to SARS-CoV-2 epitopes and were able to demonstrate that the cells responded to homologous CCC peptides with fluorospot assay [108].

Epitope mapping studies have also revealed that COVID-19 convalescent donors recognize cross-reactive T cell epitopes robustly compared to unexposed donors, suggesting that cross-reactive T cells might be enhanced after infection [74,111]. Definitive evidence that cross-reactive, meaning T cells that recognize both SARS-CoV-2 and CCCs was provided in collaboration with Dr. Kellie Smith's laboratory (Dykema et al 2021), where they expanded T cells from CCPs either with SARS-CoV-2 or HCoV-NL63 peptide pools, followed by TCR sequencing of the CD3 region of the beta chain to identify unique TCR clones and the cognate T cells that recognized and expanded as a result of antigen stimulation (viraFEST) [117]. With this assay, Dykema et al 2021 showed that CCPs do in fact harbor T cells that cross-recognize SARS-CoV-2 and CCCs.

In our study, we also characterize cross-reactive T cell responses following COVID-19 vaccinations. In chapter 3 (manuscript published in 2021), we show that cross-reactive CD4+ T cell responses to HCoV-NL63 are enhanced following COVID-19 vaccinations, suggesting that vaccinations could enhance protection to CCCs [118]. Moreover, in chapter 4 (in a manuscript published in 2022), we explore whether cross-

reactive T cells recognize peptides from bat coronaviruses not yet known to infect humans.

The function of cross-reactive T cells in protection is still not fully defined, although recent literature suggests that these T cells have functional importance. Recently, by identifying individuals during acute SARS-CoV-2 infections, Loyal et al showed that pre-existing cross-reactive T cells might be recruited and activated during COVID-19 infections, potentially allowing for faster kinetics of responses. Following vaccinations, they also showed that cross-reactive S2 domain T cells have kinetics that are reminiscent of a secondary exposure, suggesting that these T cells have functional roles during vaccination [74].

Although a majority of cross-reactive T cells are a result of cross-reactivity with endemic CCCs, there are data to suggest that not all cross-reactive T cells can be explained by cross-reactivity with CCCs. Examples include data from Loyal et al that show that unexposed individuals have pre-existing T cell responses to regions in SARS-CoV-2 that are not homologous to endemic coronaviruses. Furthermore, in their study, degree of homology did not correlate with T cell cross-reactivity [74]. These data suggest that cross-reactive T cells might arise from other unknown sources and further study is needed to elucidate the source of cross-reactivity.

1.4.3 Cross-reactive T cell mediated protection in the context of SARS-CoV-2

The identification of pre-existing and cross-reactive T cell responses begs the question of whether these responses have functional relevance in the clinical context. As discussed above, cross-reactive T cell responses have been implicated in both positive and negative clinical consequences in other diseases. There was some

concern that pre-existing SARS-CoV-2 T cell responses might influence COVID-19 vaccinations and lead to sub-optimal immune responses due to reliance of the immune response on pre-existing memory T cell responses, instead of generating de novo responses from activated naïve T cells, similar to what is described in the original antigenic sin theory [92]. Furthermore, some studies have demonstrated that cross-reactive T cell responses might have lower avidity to SARS-CoV-2 compared to CCCs [73,117]. However, despite these concerns, there is no evidence of hampered immune responses to COVID-19 vaccinations as a result of cross-reactive T cells, and robust de novo responses are generated following COVID-19 vaccinations [74].

There is a growing body of evidence to suggest that pre-existing and cross-reactive T cell responses are protective against SARS-CoV-2 infections. Recently, Swadling et al have hypothesized that cross-reactive T cells to the replication-transcription complex might enable clearance of SARS-CoV-2 infections, leading to abortive infections before seroconversion in healthcare workers [119]. Similarly, data from Kundu et al suggest that individuals with confirmed exposure to SARS-CoV-2 but remain PCR-negative have higher frequencies of cross-reactive memory T cells to nucleocapsid protein, suggesting that cross-reactive T cells might be important for rapid clearance of SARS-CoV-2 infections [100]. Cross-reactive T cells have also been hypothesized to increase protection following confirmed SARS-CoV-2 infections [100]. Sagar et al has shown that individuals with recent confirmed infections with endemic coronaviruses had less severe SARS-CoV-2 infections compared to individuals without prior endemic coronavirus infections. Collectively, these data suggest that cross-reactive T cell responses might have functional relevance and protect against SARS-

CoV-2 infections [120]. However, definitive data showing that the mechanism by which pre-existing immunity is protective are needed and likely will need to incorporate animal studies.

1.4.4 Cross-reactive antibody responses in the context of SARS-CoV-2

In contrast to T cell cross-reactivity seen between common cold coronaviruses (CCCs) and SARS-CoV-2, there have been conflicting reports of antibody cross-reactivity. Although antibodies against CCCs are found in most pre-pandemic samples, which are likely a result of prior infections, antibodies against SARS-CoV-2 in pre-pandemic serum are found in rare cases, with less than 1% of tested pre-pandemic and pre-infection serum having antibodies against SARS-CoV-2 [41]. Detected cross-reactive antibodies are more likely to be against the spike protein, and particularly against the S2 domain of the spike protein which is the most conserved domain of spike [41].

1.5 Objectives

The ongoing COVID-19 pandemic illustrates that coronaviruses pose a threat to human health. Thus, it is important to understand immune responses to these viruses following natural infections and vaccinations in different patient populations. In this thesis work, I explain work that was done to characterize T cell responses to endemic common cold coronaviruses in healthy COVID-19 unexposed individuals. Further, I describe work that characterizes SARS-CoV-2 and CCC cross-reactive T cells in COVID-19 vaccinees, and further show that cross-reactive T cells recognize peptides found in bat coronaviruses. Moreover, I show data that identifies targeted peptides by vaccine-induced T cells and assess whether T cells can recognize emerging SARS-CoV-2

variants of concern. Finally, I present work that was done to assess immune responses following COVID-19 vaccinations in people living with HIV (PLWH) in comparison to healthy donors. This work contributes to our knowledge of immune responses, with particular focus on T cell responses following COVID-19 vaccinations and T cell cross-reactivity.

1.6 References

1. Su S, Wong G, Shi W, et al. Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends Microbiol.* 2016;24(6):490-502. doi:S0966-842X(16)00071-8 [pii].

2. Cui J, Li F, Shi Z. Origin and evolution of pathogenic coronaviruses. *Nature Reviews Microbiology.* 2019;17(3):181-192. doi:10.1038/s41579-018-0118-9.

3. Verma J, Subbarao N. A comparative study of human betacoronavirus spike proteins: structure, function and therapeutics. *Arch Virol.* 2021;166(3):697-714. doi:10.1007/s00705-021-04961-y [doi].

4. Lai MM, Cavanagh D. The molecular biology of coronaviruses. *Adv Virus Res.* 1997;48:1-100. doi:S0065-3527(08)60286-9 [pii].

5. TYRRELL DA, BYNOE ML. CULTIVATION OF A NOVEL TYPE OF COMMON-COLD VIRUS IN ORGAN CULTURES. *Br Med J.* 1965;1(5448):1467-1470. doi:10.1136/bmj.1.5448.1467.

6. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med.* 1966;121(1):190-193. doi:10.3181/00379727-121-30734 [doi].

7. van der Hoek L, Pyrc K, Berkhout B. Human coronavirus NL63, a new respiratory virus. *FEMS Microbiol Rev.* 2006;30(5):760-773. doi:FMR032 [pii].

8. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol.* 2005;79(2):884-895. doi:79/2/884 [pii].

9. McIntosh K, Dees JH, Becker WB, Kapikian AZ, Chanock RM. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proc Natl Acad Sci U S A.* 1967;57(4):933-940. doi:10.1073/pnas.57.4.933 [doi].

10. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet.* 2003;361(9366):1319-1325. doi:S0140673603130772 [pii].

11. Zaki AM, van Boheemen S, Bestebroer TM, Osterhaus, Albert D. M. E., Fouchier RAM. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *N Engl J Med.* 2012;367(19):1814-1820. doi:10.1056/NEJMoa1211721.

12. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* 2020;579(7798):270-273. doi:10.1038/s41586-020-2012-7 [doi].

13. WHO COVID-19 Dashboard. WHO COVID-19 Dashboard Web site. https://covid19.who.int/. Accessed Oct 12, 2021.

14. Tao Y, Shi M, Chommanard C, et al. Surveillance of Bat Coronaviruses in Kenya Identifies Relatives of Human Coronaviruses NL63 and 229E and Their Recombination History. *J Virol.* 2017;91(5):e01953-16. Print 2017 Mar 1. doi:10.1128/JVI.01953-16 [doi].

15. Lytras S, Xia W, Hughes J, Jiang X, Robertson DL. The animal origin of SARS-CoV-2. *Science.* 2021;373(6558):968-970. doi:10.1126/science.abh0117.

16. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ. Bat-borne virus diversity, spillover and emergence. *Nature Reviews Microbiology.* 2020;18(8):461-471. doi:10.1038/s41579-020-0394-z.

17. Anthony SJ, Johnson CK, Greig DJ, et al. Global patterns in coronavirus diversity. *Virus Evol.* 2017;3(1):vex012. doi:10.1093/ve/vex012 [doi].

18. Coronaviridae Study Group of the International Committee on Taxonomy, of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature microbiology.* 2020;5(4):536-544. doi:10.1038/s41564-020-0695-z.

19. Gorbalenya AE, Baker SC, Baric RS, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology.* 2020;5(4):536-544. doi:10.1038/s41564-020-0695-z.

20. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. *J Biol Chem.* 2020;295(37):12910-12934. doi:S0021-9258(17)49954-6 [pii].

21. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature.* 2003;426(6965):450-454. doi:10.1038/nature02145.

22. Wang N, Shi X, Jiang L, et al. Structure of MERS-CoV spike receptor-binding domain complexed with human receptor DPP4. *Cell Res.* 2013;23(8):986-993. doi:10.1038/cr.2013.92 [doi].

23. Wang Q, Zhang Y, Wu L, et al. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. *Cell.* 2020;181(4):894-904.e9. doi:S0092-8674(20)30338-X [pii].

24. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nature Communications.* 2020;11(1):1620. doi:10.1038/s41467-020-15562-9.

25. Vlasak R, Luytjes W, Spaan W, Palese P. Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. *Proc Natl Acad Sci U S A.* 1988;85(12):4526-4529. doi:10.1073/pnas.85.12.4526 [doi].

26. Huang X, Dong W, Milewska A, et al. Human Coronavirus HKU1 Spike Protein Uses O-Acetylated Sialic Acid as an Attachment Receptor Determinant and Employs

Hemagglutinin-Esterase Protein as a Receptor-Destroying Enzyme. *J Virol.* 2015;89(14):7202-7213. doi:10.1128/JVI.00854-15 [doi].

27. Yeager CL, Ashmun RA, Williams RK, et al. Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature.* 1992;357(6377):420-422. doi:10.1038/357420a0.

28. Heike H, Krzysztof P, van der HL, Martina G, Ben B, Pöhlmann Stefan. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proceedings of the National Academy of Sciences.* 2005;102(22):7988-7993. doi:10.1073/pnas.0409465102.

29. Tregoning JS, Flight KE, Higham SL, Wang Z, Pierce BF. Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nature Reviews Immunology.* 2021;21(10):626-636. doi:10.1038/s41577-021-00592-1.

30. World Health Organization. Tracking SARS-CoV-2 variants. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.

31. Morens DM, Taubenberger JK, Fauci AS. Universal Coronavirus Vaccines — An Urgent Need. *N Engl J Med.* 2021. doi:10.1056/NEJMp2118468.

32. Wang Q, Qi J, Yuan Y, et al. Bat origins of MERS-CoV supported by bat coronavirus HKU4 usage of human receptor CD26. *Cell Host Microbe.* 2014;16(3):328-337. doi:S1931-3128(14)00301-1 [pii].

33. Yang Y, Du L, Liu C, et al. Receptor usage and cell entry of bat coronavirus HKU4 provide insight into bat-to-human transmission of MERS coronavirus. *Proc Natl Acad Sci U S A.* 2014;111(34):12516-12521. doi:10.1073/pnas.1405889111 [doi].

34. Hu B, Zeng L, Yang X, et al. Discovery of a rich gene pool of bat SARS-related coronaviruses provides new insights into the origin of SARS coronavirus. *PLOS Pathogens.* 2017;13(11):e1006698. https://doi.org/10.1371/journal.ppat.1006698.

35. Menachery VD, Yount BL, Debbink K, et al. A SARS-like cluster of circulating bat coronaviruses shows potential for human emergence. *Nat Med.* 2015;21(12):1508-1513. doi:10.1038/nm.3985.

36. Ge X, Li J, Yang X, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. *Nature.* 2013;503(7477):535-538. doi:10.1038/nature12711.

37. Li LL, Wang JL, Ma XH, et al. A novel SARS-CoV-2 related coronavirus with complex recombination isolated from bats in Yunnan province, China. *Emerg Microbes Infect.* 2021;10(1):1683-1690. doi:10.1080/22221751.2021.1964925 [doi].

38. Flemming A. Omicron, the great escape artist. *Nature Reviews Immunology.* 2022;22(2):75. doi:10.1038/s41577-022-00676-6.

39. Tao K, Tzou PL, Nouhin J, et al. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nature Reviews Genetics.* 2021;22(12):757-773. doi:10.1038/s41576-021-00408-x.

40. Kenneth M. Murphy, Casey Weaver. *Janeway's Immunobiology .* W. W. Norton & Company; 2016.

41. Röltgen K, Boyd SD. Antibody and B cell responses to SARS-CoV-2 infection and vaccination. *Cell Host Microbe.* 2021;29(7):1063-1075. doi:S1931-3128(21)00287-0 [pii].

42. Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell.* 2021;184(4):861-880. doi:S0092-8674(21)00007-6 [pii].

43. Cohen KW, Linderman SL, Moodie Z, et al. Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. *Cell Rep Med.* 2021;2(7):100354. doi:10.1016/j.xcrm.2021.100354 [doi].

44. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science.* 2021;371(6529):eabf4063. doi:10.1126/science.abf4063.

45. Hall VJ, Foulkes S, Charlett A, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). *The Lancet.* 2021;397(10283):1459-1469. doi:10.1016/S0140-6736(21)00675-9.

46. Edridge AWD, Kaczorowska J, Hoste ACR, et al. Seasonal coronavirus protective immunity is short-lasting. *Nat Med.* 2020;26(11):1691-1693. doi:10.1038/s41591-020-1083-1.

47. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol Infect.* 1990;105(2):435-446. doi:10.1017/s0950268800048019.

48. Sariol A, Perlman S. Lessons for COVID-19 Immunity from Other Coronavirus Infections. *Immunity.* 2020;53(2):248-263. doi:10.1016/j.immuni.2020.07.005.

49. Wu L, Wang N, Chang Y, et al. Duration of antibody responses after severe acute respiratory syndrome. *Emerging infectious diseases.* 2007;13(10):1562-1564. doi:10.3201/eid1310.070576.

50. Tang F, Quan Y, Xin ZT, et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. *J Immunol.* 2011;186(12):7264-7268. doi:10.4049/jimmunol.0903490 [doi].

51. Rydyznski Moderbacher C, Ramirez SI, Dan JM, et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell.* 2020;183(4):996-1012.e19. doi:https://doi.org/10.1016/j.cell.2020.09.038.

52. Nelde A, Bilich T, Heitmann JS, et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat Immunol.* 2021;22(1):74-85. doi:10.1038/s41590-020-00808-x.

53. Bergamaschi L, Mescia F, Turner L, et al. Longitudinal analysis reveals that delayed bystander CD8+ T cell activation and early immune pathology distinguish severe COVID-19 from mild disease. *Immunity.* 2021;54(6):1257-1275.e8. doi:https://doi.org/10.1016/j.immuni.2021.05.010.

54. Notarbartolo S, Ranzani V, Bandera A, et al. Integrated longitudinal immunophenotypic, transcriptional and repertoire analyses delineate immune responses in COVID-19 patients. *Sci Immunol.* 2021;6(62):eabg5021. doi: 10.1126/sciimmunol.abg5021. doi:eabg5021 [pii].

55. Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol.* 2022;23(2):186-193. doi:10.1038/s41590-021-01122-w.

56. Zhou R, To KK, Wong Y, et al. Acute SARS-CoV-2 Infection Impairs Dendritic Cell and T Cell Responses. *Immunity.* 2020;53(4):864-877.e5. doi:https://doi.org/10.1016/j.immuni.2020.07.026.

57. Gallais F, Velay A, Nazon C, et al. Intrafamilial Exposure to SARS-CoV-2 Associated with Cellular Immune Response without Seroconversion, France. *Emerging infectious diseases.* 2021;27(1):113-121. doi:10.3201/eid2701.203611.

58. Wang Z, Yang X, Zhong J, et al. Exposure to SARS-CoV-2 generates T-cell memory in the absence of a detectable viral infection. *Nature Communications.* 2021;12(1):1724. doi:10.1038/s41467-021-22036-z.

59. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. *Cell.* 2020;183(1):158-168.e14. doi:S0092-8674(20)31008-4 [pii].

60. Cohen B, Rubinstein R, Gans MD, Deng L, Rubinstein A, Eisenberg R. COVID-19 infection in 10 common variable immunodeficiency patients in New York City. *J Allergy Clin Immunol Pract.* 2021;9(1):504-507.e1. doi:S2213-2198(20)31225-3 [pii].

61. Bange EM, Han NA, Wileyto P, et al. CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat Med.* 2021;27(7):1280-1289. doi:10.1038/s41591-021-01386-7.

62. Jones JM, Faruqi AJ, Sullivan JK, Calabrese C, Calabrese LH. COVID-19 Outcomes in Patients Undergoing B Cell Depletion Therapy and Those with Humoral Immunodeficiency States: A Scoping Review. *Pathog Immun.* 2021;6(1):76-103. doi:10.20411/pai.v6i1.435 [doi].

63. Zhao J, Zhao J, Mangalam AK, et al. Airway Memory CD4+ T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity.* 2016;44(6):1379-1391. doi:https://doi.org/10.1016/j.immuni.2016.05.006.

64. Zhao J, Zhao J, Perlman S. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. *J Virol.* 2010;84(18):9318-9325. doi:10.1128/JVI.01049-10 [doi].

65. Rudragouda C, Craig F, Jincun Z, Meyerholz David K, Stanley P, Sandri-Goldin RM. Virus-Specific Memory CD8 T Cells Provide Substantial Protection from Lethal Severe Acute Respiratory Syndrome Coronavirus Infection. *J Virol.* 2014;88(19):11034-11044. doi:10.1128/JVI.01505-14.

66. Jincun Z, Kun L, Wohlford-Lenane Christine, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. *Proceedings of the National Academy of Sciences.* 2014;111(13):4970-4975. doi:10.1073/pnas.1323279111.

67. Israelow B, Mao T, Klein J, et al. Adaptive immune determinants of viral clearance and protection in mouse models of SARS-CoV-2. *Sci Immunol.* 2021;6(64):eabl4509. doi:10.1126/sciimmunol.abl4509.

68. Zhuang Z, Lai X, Sun J, et al. Mapping and role of T cell response in SARS-CoV-2–infected mice. *J Exp Med.* 2021;218(4):e20202187. doi:10.1084/jem.20202187.

69. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature.* 2021;590(7847):630-634. doi:10.1038/s41586-020-03041-6.

70. Juno JA, Tan H, Lee WS, et al. Humoral and circulating follicular helper T cell responses in recovered patients with COVID-19. *Nat Med.* 2020;26(9):1428-1434. doi:10.1038/s41591-020-0995-0.

71. Grifoni A, Sidney J, Vita R, et al. SARS-CoV-2 human T cell epitopes: Adaptive immune response against COVID-19. *Cell Host & Microbe.* 2021;29(7):1076-1092. doi:10.1016/j.chom.2021.05.010.

72. Peng Y, Felce SL, Dong D, et al. An immunodominant NP105–113-B*07:02 cytotoxic T cell response controls viral replication and is associated with less severe COVID-19 disease. *Nat Immunol.* 2022;23(1):50-61. doi:10.1038/s41590-021-01084-z.

73. Bacher P, Rosati E, Esser D, et al. Low-Avidity CD4(+) T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19. *Immunity.* 2020;53(6):1258-1271.e5. doi:S1074-7613(20)30503-3 [pii].

74. Loyal L, Braun J, Henze L, et al. Cross-reactive $CD4^+$ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science.* 2021;374(6564):eabh1823. doi:10.1126/science.abh1823.

75. Lu Z, Laing ED, Pena DaMata J, et al. Durability of SARS-CoV-2-Specific T-Cell Responses at 12 Months Postinfection. *J Infect Dis.* 2021;224(12):2010-2019. doi:10.1093/infdis/jiab543 [doi].

76. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature.* 2020;584(7821):457-462. doi:10.1038/s41586-020-2550-z [doi].

77. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med.* 2020;383(27):2603-2615. doi:10.1056/NEJMoa2034577.

78. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med.* 2021;384(5):403-416. doi:10.1056/NEJMoa2035389.

79. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature.* 2020;586(7830):594-599. doi:10.1038/s41586-020-2814-7.

80. Kedzierska K, Thomas PG. Count on us: T cells in SARS-CoV-2 infection and vaccination. *Cell Reports Medicine.* 2022;3(3):100562. doi:https://doi.org/10.1016/j.xcrm.2022.100562.

81. Mudd PA, Minervina AA, Pogorelyy MV, et al. SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans. *Cell.* 2022;185(4):603-613.e15. doi:S0092-8674(21)01489-6 [pii].

82. Zhang Z, Mateus J, Coelho CH, et al. Humoral and cellular immune memory to four COVID-19 vaccines. *bioRxiv.* 2022:2022.03.18.484953. doi:10.1101/2022.03.18.484953.

83. Rosenberg ES, Dorabawila V, Easton D, et al. Covid-19 Vaccine Effectiveness in New York State. *N Engl J Med.* 2022;386(2):116-127. doi:10.1056/NEJMoa2116063.

84. Levin EG, Lustig Y, Cohen C, et al. Waning Immune Humoral Response to BNT162b2 Covid-19 Vaccine over 6 Months. *N Engl J Med.* 2021. doi:10.1056/NEJMoa2114583.

85. Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. *Science.* 2021;374(6572):abm0829. doi:10.1126/science.abm0829.

86. Tartof SY, Slezak JM, Fischer H, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *The Lancet.* 2021;398(10309):1407-1416. doi:10.1016/S0140-6736(21)02183-8.

87. Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature.* 1988;334(6181):395-402. doi:10.1038/334395a0.

88. Danilova L, Anagnostou V, Caushi JX, et al. The Mutation-Associated Neoantigen Functional Expansion of Specific T Cells (MANAFEST) Assay: A Sensitive Platform for Monitoring Antitumor Immunity. *Cancer Immunol Res.* 2018;6(8):888-899. doi:10.1158/2326-6066.CIR-18-0129 [doi].

89. Mason D. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol Today.* 1998;19(9):395-404. doi:https://doi.org/10.1016/S0167-5699(98)01299-7.

90. Sewell AK. Why must T cells be cross-reactive? *Nature Reviews Immunology.* 2012;12(9):669-677. doi:10.1038/nri3279.

91. Petrova G, Ferrante A, Gorski J. Cross-reactivity of T cells and its role in the immune system. *Crit Rev Immunol.* 2012;32(4):349-372. doi:10.1615/critrevimmunol.v32.i4.50.

92. Sette A, Crotty S. Pre-existing immunity to SARS-CoV-2: the knowns and unknowns. *Nature Reviews Immunology.* 2020;20(8):457-458. doi:10.1038/s41577-020-0389-z.

93. Yin Y, Mariuzza RA. The Multiple Mechanisms of T Cell Receptor Cross-reactivity. *Immunity.* 2009;31(6):849-851. doi:https://doi.org/10.1016/j.immuni.2009.12.002.

94. Lee CH, Salio M, Napolitani G, Ogg G, Simmons A, Koohy H. Predicting Cross-Reactivity and Antigen Specificity of T Cell Receptors. *Frontiers in Immunology.* 2020;11. https://www.frontiersin.org/article/10.3389/fimmu.2020.565096.

95. Stewart AJ, Devlin PM. The history of the smallpox vaccine. *J Infect.* 2006;52(5):329-334. doi:https://doi.org/10.1016/j.jinf.2005.07.021.

96. Agrawal B. Heterologous Immunity: Role in Natural and Vaccine-Induced Resistance to Infections. *Frontiers in Immunology.* 2019;10. https://www.frontiersin.org/article/10.3389/fimmu.2019.02631.

97. Sridhar S, Begom S, Bermingham A, et al. Cellular immune correlates of protection against symptomatic pandemic influenza. *Nat Med.* 2013;19(10):1305-1312. doi:10.1038/nm.3350.

98. Wilkinson TM, Li CKF, Chui CSC, et al. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. *Nat Med.* 2012;18(2):274-280. doi:10.1038/nm.2612.

99. Mizgerd, Manish Sagar AND Katherine Reifler AND Michael Rossi AND Nancy S. Miller AND Pranay Sinha AND Laura F. White AND Joseph P. Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest.* 2021;131(1). doi:10.1172/JCI143380.

100. Kundu R, Narean JS, Wang L, et al. Cross-reactive memory T cells associate with protection against SARS-CoV-2 infection in COVID-19 contacts. *Nature Communications.* 2022;13(1):80. doi:10.1038/s41467-021-27674-x.

101. Eggenhuizen PJ, Ng BH, Chang J, et al. BCG Vaccine Derived Peptides Induce SARS-CoV-2 T Cell Cross-Reactivity. *Frontiers in Immunology.* 2021;12. https://www.frontiersin.org/article/10.3389/fimmu.2021.692729.

102. Tarabini RF, Rigo MM, Faustino Fonseca A, et al. Large-Scale Structure-Based Screening of Potential T Cell Cross-Reactivities Involving Peptide-Targets From BCG Vaccine and SARS-CoV-2. *Frontiers in Immunology.* 2022;12. https://www.frontiersin.org/article/10.3389/fimmu.2021.812176.

103. Vatti A, Monsalve DM, Pacheco Y, Chang C, Anaya JM, Gershwin ME. Original antigenic sin: A comprehensive review. *J Autoimmun.* 2017;83:12-21. doi:S0896-8411(17)30222-6 [pii].

104. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. *Nature Reviews Immunology.* 2011;11(8):532-543. doi:10.1038/nri3014.

105. Agrawal B. Heterologous Immunity: Role in Natural and Vaccine-Induced Resistance to Infections. *Frontiers in Immunology.* 2019;10. https://www.frontiersin.org/article/10.3389/fimmu.2019.02631.

106. Linette GP, Stadtmauer EA, Maus MV, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood.* 2013;122(6):863-871. doi:10.1182/blood-2013-03-490565.

107. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell.* 2020;181(7):1489-1501.e15. doi:S0092-8674(20)30610-3 [pii].

108. Mateus J, Grifoni A, Tarke A, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science.* 2020;370(6512):89-94. doi:10.1126/science.abd3871 [doi].

109. Braun J, Loyal L, Frentsch M, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature.* 2020;587(7833):270-274. doi:10.1038/s41586-020-2598-9.

110. Meckiff BJ, Ramírez-Suástegui C, Fajardo V, et al. Imbalance of Regulatory and Cytotoxic SARS-CoV-2-Reactive CD4+ T Cells in COVID-19. *Cell.* 2020;183(5):1340-1353.e16. doi:https://doi.org/10.1016/j.cell.2020.10.001.

111. Nelde A, Bilich T, Heitmann JS, et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat Immunol.* 2021;22(1):74-85. doi:10.1038/s41590-020-00808-x.

112. Killerby ME, Biggs HM, Haynes A, et al. Human coronavirus circulation in the United States 2014–2017. *Journal of Clinical Virology.* 2018;101:52-56. doi:https://doi.org/10.1016/j.jcv.2018.01.019.

113. Severance Emily G, Ioannis B, Dickerson Faith B, et al. Development of a Nucleocapsid-Based Human Coronavirus Immunoassay and Estimates of Individuals Exposed to Coronavirus in a U.S. Metropolitan Population. *Clinical and Vaccine Immunology.* 2008;15(12):1805-1810. doi:10.1128/CVI.00124-08.

114. Galanti M, Shaman J. Direct Observation of Repeated Infections With Endemic Coronaviruses. *J Infect Dis.* 2021;223(3):409-415. doi:10.1093/infdis/jiaa392 [doi].

115. Woldemeskel BA, Kwaa AK, Garliss CC, Laeyendecker O, Ray SC, Blankson JN. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. *J Clin Invest.* 2020;130(12):6631-6638. doi:143120 [pii].

116. Yu ED, Narowski TM, Wang E, et al. Immunological memory to Common Cold Coronaviruses assessed longitudinally over a three-year period. *bioRxiv.* 2022:2022.03.01.482548. doi:10.1101/2022.03.01.482548.

117. Dykema AG, Zhang B, Woldemeskel BA, et al. Functional characterization of CD4+ T cell receptors crossreactive for SARS-CoV-2 and endemic coronaviruses. *J Clin Invest.* 2021;131(10):e146922. doi: 10.1172/JCI146922. doi:146922 [pii].

118. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. *J Clin Invest.* 2021;131(10):e149335. doi: 10.1172/JCI149335. doi:149335 [pii].

119. Swadling L, Diniz MO, Schmidt NM, et al. Pre-existing polymerase-specific T cells expand in abortive seronegative SARS-CoV-2. *Nature.* 2022;601(7891):110-117. doi:10.1038/s41586-021-04186-8.

120. Sagar M, Reifler K, Rossi M, et al. Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest.* 2021;131(1). doi:10.1172/JCI143380.

121. Woldemeskel BA, Garliss CC, Blankson JN. mRNA Vaccine-Elicited Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)–Specific T Cells Persist at 6

Months and Recognize the Delta Variant. *Clin Infect Dis.* 2021:ciab915. doi:10.1093/cid/ciab915.

122. Woldemeskel BA, Dykema AG, Garliss C, Cherfils S, Smith KN, Blankson JN. CD4+ T-cells from COVID-19 mRNA vaccine recipients recognize a conserved epitope present in diverse coronaviruses. *J Clin Invest.* 2022. doi:10.1172/JCI156083.

123. Tseng HF, Ackerson BK, Luo Y, et al. Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. *Nat Med.* 2022. doi:10.1038/s41591-022-01753-y.

124. Collie S, Champion J, Moultrie H, Bekker L, Gray G. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. *N Engl J Med.* 2022;386(5):494-496. doi:10.1056/NEJMc2119270.

125. Woldemeskel B, Garliss C, Aytenfisu T, et al. SARS-CoV-2 -specific immune responses in boosted vaccine recipients with breakthrough infections during the Omicron variant surge. (accepted for publication).

126. GeurtsvanKessel CH, Geers D, Schmitz KS, et al. Divergent SARS-CoV-2 Omicron–reactive T and B cell responses in COVID-19 vaccine recipients. *Sci Immunol.* 2022;7(69):eabo2202. doi:10.1126/sciimmunol.abo2202.

127. Jincun Z, Jingxian Z, Stanley P. T Cell Responses Are Required for Protection from Clinical Disease and for Virus Clearance in Severe Acute Respiratory Syndrome Coronavirus-Infected Mice. *J Virol.* 2010;84(18):9318-9325. doi:10.1128/JVI.01049-10.

128. Garcia-Beltran W, Lam EC, St Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell.* 2021;184(9):2372-2383.e9. doi:10.1016/j.cell.2021.03.013.

129. Heitmann JS, Bilich T, Tandler C, et al. A COVID-19 peptide vaccine for the induction of SARS-CoV-2 T cell immunity. *Nature.* 2022;601(7894):617-622. doi:10.1038/s41586-021-04232-5.

130. Dolgin E. T-cell vaccines could top up immunity to COVID, as variants loom large. Nature Biotechnology News Web site. https://www.nature.com/articles/d41587-021-00025-3. Updated 2022.

2 Chapter 2: Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2

2.1 Abstract

T cell responses to the common cold coronaviruses have not been well characterized. Pre-existing T cell immunity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reported, but a recent study suggested that this immunity was due to cross-recognition of the novel coronavirus by T cells specific for the common cold coronaviruses. We used the enzyme-linked immunospot (ELISpot) assay to characterize the T cell responses against peptide pools derived from the spike protein of 3 common cold coronaviruses (HCoV-229E, HCoV-NL63, HCoV-OC43) and SARS-

CoV-2 in 21 healthy donors (HDs) who were seronegative for SARS-CoV-2 and had no known exposure to the virus. An in vitro expansion culture assay was also used to analyze memory T cell responses. We found responses to the spike protein of the 3 common cold coronaviruses in many of the donors. We then focused on HCoV-NL63 and detected broad T cell responses to the spike protein and identified 22 targeted peptides. Interestingly, only 1 study participant had a significant response to SARS-CoV-2 spike or nucleocapsid protein in the ELIspot assay. In vitro expansion studies suggested that T cells specific for the HCoV-NL63 spike protein in this individual could also recognize SARS-CoV-2 spike protein peptide pools. HDs have circulating T cells specific for the spike proteins of HCoV-NL63, HCoV-229E and HCoV-OC43. T cell responses to SARS-CoV-2 spike and nucleocapsid proteins were present in only 1 participant and were potentially the result of cross-recognition by T cells specific of the common cold coronaviruses. Further studies are needed to determine whether this cross-recognition influences coronaviruses disease 2019 (COVID-19) outcomes.

2.2 Introduction

There are 4 known human common cold coronaviruses (HCoV) that cause mild respiratory disease: HCoV-NL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1 (1). Seroprevalence studies show that a large percentage of adults have been exposed to these viruses (2). Interestingly, surveillance studies have shown that reinfection with these viruses can occur (3, 4), suggesting that immunity is only partially protective. This theory is supported by a challenge study showing that study participants with lower titers of antibodies against HCoV-229E were infected and developed symptoms following experimental inoculation with the

virus (5). Some of the same individuals could be reinfected by the same virus 1 year later, but they experienced minimal symptoms and had reduced periods of viral shedding (5). Despite these data, the T cell responses to these viruses in healthy donors (HDs) have not been characterized in an unbiased manner, and it is not known whether T cells con- tribute to the partial immunity described above.

Severe acute respiratory syndrome coronavirus 2–specific (SARS-CoV-2–specific) T cell responses have been detected in patients with coronavirus disease 2019 (COVID-19) (6–20), and while T cell responses against SARS-CoV have been shown to be long lasting (8), it is not yet known whether SARS-CoV-2–specific T cells will confer protection against reinfection. Recent studies have suggested that preexisting T cell immunity to SARS-CoV-2 is present in some unexposed, HDs (6–11). However, other studies have found no evidence of SARS-CoV-2–specific T cells in unexposed individuals (13, 21).

In this study, we sought to characterize the T cell responses to human cold coronaviruses and to determine whether preexisting immunity to SARS-CoV-2 was due to cross-recognition by T cells specific for endemic coronaviruses. To do this, we examined T cell responses to the spike (S) protein of 3 of the 4 common cold coronaviruses (HCoV-NL63, HCoV-229E, and HCoV-OC43) and to SARS-CoV-2 in HDs with no known exposure to SARS-CoV-2. We then focused on HCoV-NL63 and identified what we believe to be 19 novel targeted peptides. We also examined the responses to the SARS-CoV-2 nucleocapsid (N) and membrane (M) proteins and performed experiments to deter- mine whether T

cell cross-recognition of HCoV-NL63 and SARS-CoV-2 S peptides was possible. We believe our results further the understanding of the immune response to coronaviruses and may have implications for SARS-CoV-2 vaccine trials.

2.3 Methods

Subjects

Blood samples from healthy laboratory donors and 4 individuals who recovered from COVID-19 were obtained between April and July 2020. All the HDs reported no known exposure to COVID-19 patients and no upper respiratory tract infections over the preceding 3 months. Twelve of the HDs were between the ages of 20 and 29 years, 3 were between the ages of 30 and 39 years, 5 were between the ages of 40 and 49 years, and 1 was between the ages of 50 and 59 years. Thirteen of the HDs were men and 8 were women. We also studied 4 patients who had recovered from COVID-19. Blood was drawn 3 months after the onset of their symptoms. Three were previously healthy and had mild disease courses (CCP1, CCP3, CCP4), and 1 participant with well-controlled HIV-1 infection on antiretroviral therapy had a severe disease course (CCP2). For all experiments, PBMCs were collected from whole blood after Ficoll-Paque PLUS gradient centrifugation (GE Healthcare Life Sciences). For some experiments, $CD8^+$ T cells were depleted using Miltenyi Biotec $CD8^+$ T Cell Positive Selection Kits. High-resolution class II typing was performed on PBMCs from 6 HDs at the Johns Hopkins Hospital Immunogenetics Laboratory.

The Immune Epitope Database and Analysis Resource (http://www.iedb.org) was used for optimal epitope and HLA-binding predictions using recommended parameters (27).

Peptides and ELISPOT assays

Peptides for the S protein of HCoV-NL63, HCoV-229E, HCoV-OC43, and SARS-Cov-2, as well as the M and N proteins of SARS-CoV-2 were obtained from BEI Resources and were reconstituted with DMSO at a concentration of 10 mg/ml. The HCoV-229E S protein peptide pool has 195 peptides consisting of 17 mer with 11 amino acid overlaps. The HCoV-NL63 S protein peptide pool has 226 peptides made up of 14–17 mer with 11–13 amino acid overlaps. The HCoV-OC43 S protein peptide pool has 226 peptides made up of 17 or 18 mer with 11 amino acid overlaps. The SARS-CoV-2 peptides are 12 mer, 13 mer, or 17 mer, with 10 amino acid overlaps. The S protein peptide pool was made up of 181 peptides, the N protein peptide pool was made up of 59 peptides, and the M peptide pool was made up of 31 peptides. All the peptides were combined into 1 pool for each viral protein. Pools of 10 peptides were made for the HCoV-NL63 S protein, and 1 pool had 17 peptides. Peptides for CEF were obtained from Anaspec. The pool consisted of thirty-two 8–12 mer peptides. Stimulation with anti-CD3 antibody (Mabtech, 1 μg/mL) was used as a positive control for each study participant.

IFN-γ ELISPOT assays were performed as previously described (28, 29). Briefly ELISPOT Pro and ELISPOT Plus kits with precoated plates were

purchased from Mabtech. The wells were plated with unfractionated PBMCs or $CD8^+$ T cell–depleted PBMCs at 250,000 cells/well, and the cells were cultured for 22–24 hours with HCoV pep- tides at a concentration of 10 μg/mL or with CEF peptides at a con- centration of 3 μg/mL. The plates were then processed according to the manufacturer's protocol and read by a blinded independent investigator using an automated reading system. Four replicates per pool were run for the comparison of the different viral proteins. The replicate furthest from the median was not used. If 2 values were equally distant from the median, then the higher value was discarded. Two replicates were run for the HCoV-NL63 S protein pools that examined the breadth of the T cell responses. For epitope mapping, each individual peptide present in a pool was tested in duplicate wells. A peptide was only considered to be positive if both wells had values that were at least twice the average of the untreated wells and the average stimulation index was above 3 and more than 20 SFU/10^6 cells were present.

Expansion culture assay

PBMCs (10^7 cells) were cultured in R10 media with 10 U/mL IL-2 and 5 μg/mL peptides for 10–12 days in a modified version of a previously described assay (22). The media were not changed during this period. The cells were then washed and replated in fresh R10 with 10 U/mL IL-2 and rested 1 day before they were stimulated again with 5 μg/mL peptide with protein transport inhibitors (GolgiPlug, 1 μg/mL; GolgiStop, 0.7 μg/mL) as well as an antibody against

CD107a (FITC, clone H4A3) and antibodies against CD28 and CD49d (all from BD Biosciences). After a 12-hour incubation, the cells were washed and stained with annexin V (BV-421, BD Biosciences, 563973) and antibodies against CD3 (APC-Cy-7, Bio- Legend, 300426), CD4 (PerCP-CY-5.5, BioLegend, 300530), CD8 (BV-605, BioLegend, 301040), and CD107a (FITC, BD Biosciences, 555800). The cells were then fixed, permeabilized, and stained intra- cellularly for the following cytokines: TNF-α (PE-Cy-7, BD Biosciences, 557647), IFN-γ (APC, BD Biosciences, 506510), and IL-2 (PE, BioLegend, 500307). Flow cytometry was performed on a BD FACS LSR Fortessa flow cytometer, and data were analyzed using FlowJo, version 10. Data on a minimum of 100,000 events in the lymphocyte gate were collected and analyzed.

Serology

Donors were tested for SARS-CoV-2–specific antibodies with a rapid IgG/IgM combined antibody prescreening kit (sensing. self). Plasma from HD9 was also tested for SARS-CoV-2 IgG and IgA antibodies at the Johns Hopkins Hospital clinical laboratory to confirm seronegative status.

Statistics

All statistical analyses were performed using GraphPad Prism 8.4.3 (GraphPad Software). For experiments requiring multiple comparisons, a 1-way ANOVA with Geisser-Greenhouse correction was used. Dunnett's multiple-comparison test was used to determine difference between groups. For experiments requiring

comparisons between 2 groups, a 2-tailed, paired Student's t test was used to determine significance. A P value of less than 0.05 was considered statistically significant. Study approval. The study was approved by the IRB of Johns Hopkins University. Written informed consent was obtained from all study participants prior to their inclusion in the study.

2.4 Results

Healthy donors (HDs) have circulating CD4$^+$ T cell responses to 3 common cold coronaviruses but not to SARS-CoV-2.

Healthy donors (HDs) refer to individuals not previously exposed to SARS-Cov-2. To quantify responses in HDs, we performed IFN-γ ELISPOT assays to measure the frequency of T cells that secreted IFN-γ in response to peptides from the S protein from the common cold coronaviruses and SARS-CoV-2. A stimulation index (SI) was calculated by dividing the spot-forming units (SFU) per million PBMCs elicited by a peptide pool by the SFU present in wells treated with media alone. A positive response was defined as SI of greater than or equal to 3 and an absolute value of greater than or equal to 20 SFU per million PBMCs. The median frequency of T cells reactive to HCoV-NL63, HCoV-229E, and HCoV-OC43 S proteins was 33, 23, and 21 cells per million PBMCs, respectively. In contrast, the median response to SARS-CoV-2 was just 3 T cells per million PBMCs, which was not statistically different from the response to media alone (Figure 2.1A). Of the 21 HDs tested, 15, 10, and 10 individuals met both criteria for positive responses to HCoV-NL63, HCoV-229E, and HCoV-OC43 S peptides, respectively, whereas only 1 HD (HD9, indicated by

the arrowheads in Figure 2.1) met both criteria for a positive response to the SARS- CoV-2 S peptide pool (Figure 2.1B).

In order to determine whether $CD4^+$ or $CD8^+$ T cells were responding to the peptides, we depleted $CD8^+$ T cells from PBMCs and used the residual cells in an ELISpot assay. In virtually all study participants, $CD8^+$ T cell depletion increased the number of SFU in all conditions. The median responses elicited by HCoV-NL63, HCoV-229E, and HCoV-OC43 S peptide pools were 61, 41, and 31 SFU per million cells, respectively (Figure 2.1C), and while the median responses to the SARS-CoV-2 S peptide pools were also higher, none of the participants met both the absolute count and the SI criteria for a positive response (Figure 2.1, C and D). In contrast, T cells from COVID-19 convalescent patients (CCPs) recognized peptide pools from the SARS-CoV-2 S protein (Figure 2.1, E-H). The increase in responses to the common cold coronavirus S peptide pools following $CD8^+$ T cell depletion suggests that $CD4^+$ T cells were the major effector cells in our assay, especially since depletion of $CD8^+$ T cells abrogated the responses to MHC class I–restricted peptide pools from CMV, EBV, and influenza (CEF) (Figure 2.2). However, it is likely that $CD4^+$ T cells were more apt to be induced by the relatively long peptides used in our assay.

We then asked whether HD T cells were better able to recognize other SARS-CoV-2 peptides, including those from the N and M proteins. As shown in Figure 2.1, I and J, although the majority of HDs responded to CEF peptides, only HD9 had a robust response to peptides from the N protein, and no

individual responded to peptides from the M protein. Although some of the other HDs had T cell responses that met the criteria for a positive response according to the SI, the absolute number of responding cells was less than the 20 SFU cutoff. In contrast, T cells from 2 of the 4 CCPs recognized peptide pools from the SARS-CoV-2 M and N proteins (Figure 2.1, K and L).

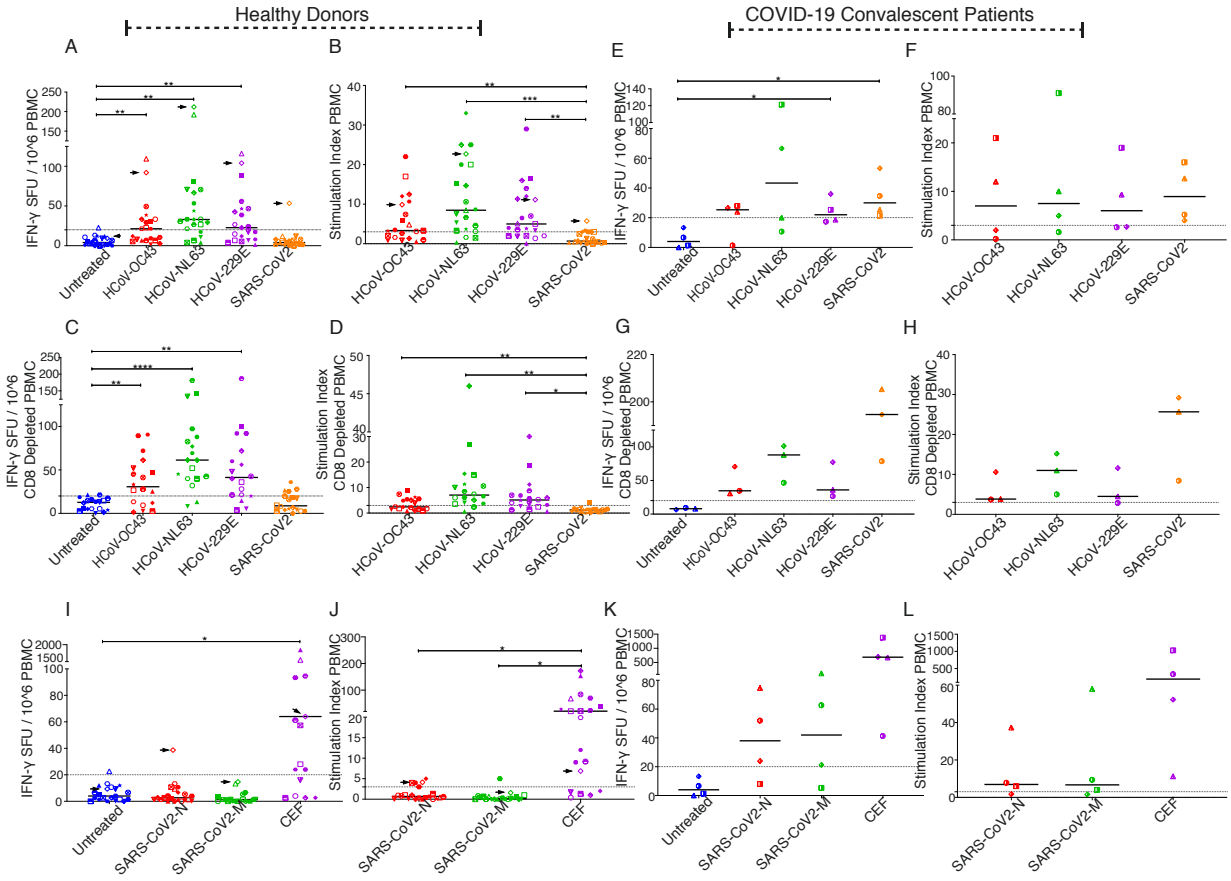

Figure 2.1: IFN-γ responses to viral peptide pools from HDs and CCPs. The number of SFU from unfractioned PBMCs (A and E) and CD8+ T cell–depleted PBMCs (C and G) and the corresponding stimulation indices (B, D, F, and H) in response to S protein peptide pools from different viruses are shown. The number of SFU (I and K)

and the stimulation indices (J and L) from unfractioned PBMCs in response to CEF and SARS-CoV-2 M and N peptide pools are also shown. Arrows indicate HD9. Each data point represents the mean of 3 replicate values. Horizontal bars represent the median. Statistical comparisons were performed using 1-way ANOVA with Geisser-Greenhouse correction and Dunnett's multiple-comparison test (n = 19–21 for samples from HDs; n = 3–4 for samples from patients with COVID-19). *P = 0.0332, **P = 0.0021, ***P = 0.0002, and ****P < 0.0001.

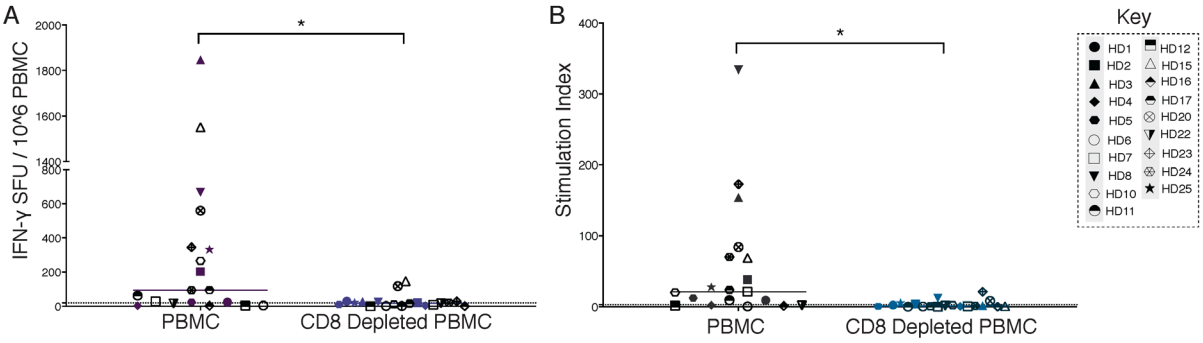

Figure 2.2: INF-γ responses to CEF and unfractioned PBMCs and CD8 Depleted PBMCs. The number of spot forming units (SFU) from unfractioned PBMCs and CD8+ T cell depleted PBMCs (A), and corresponding stimulation indices (B) in response to CEF are shown (n=19). Each data point represents the mean of three replicate values. Horizontal bars represent the median. Statistical analysis was performed with a two-tailed, paired student's t-test.

2.4.2. T cells target various regions of the HCoV-NL63 S protein.

The most robust T cell responses were directed against the S protein of HCoV-NL63, so we focused on this virus for epitope-mapping studies. In order to determine which regions of the S protein were targeted by HD T cells, we performed ELISPOT assays with sequential peptide pools consisting of 10 overlapping peptides. As shown in Figure 2.3, we observed broad responses to the S peptide pools, and every pool was targeted by T cells from at least 1 individual. However, the most potent responses were elicited by pools 14 (amino acids 777–847), 2 (amino acids 61–131), and 15 (amino acids 837–907), with a median of 36, 28, and 26 T cells producing IFN-γ, respectively (marked in red on Figure 2.3.) In order to define the targeted peptides, we repeated the ELISpot assay with individual peptides from the pools that were targeted by the 6 HDs for whom we had sufficient numbers of PBMCs. Table 2.1 contains the list of the 22 peptides we were able to identify and the potential optimal epitopes and restricting HLA alleles. Interestingly, peptides 16 (amino acids 91–107), 132 (amino acids 783–799), and 141 (amino acids 837–853) were each targeted in 2 individuals.

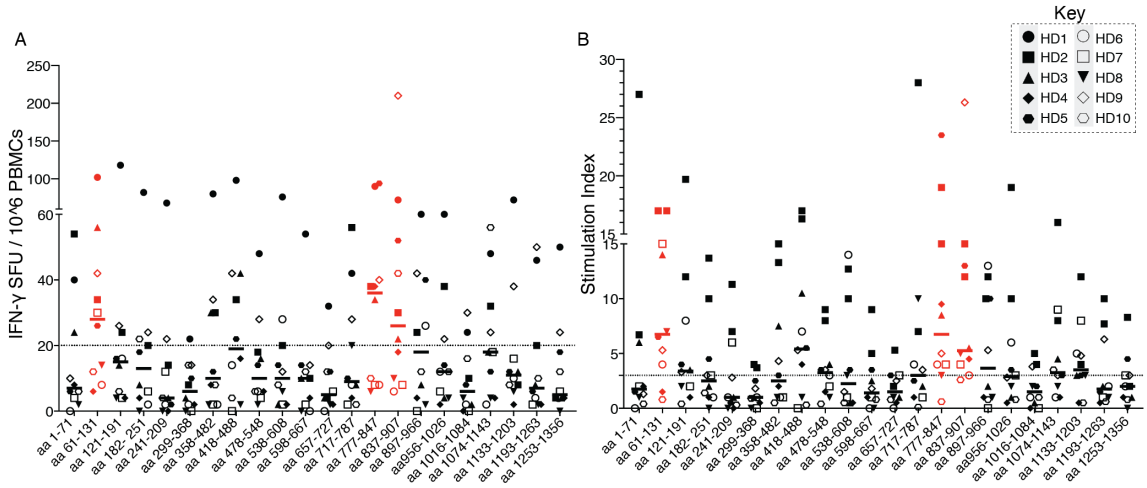

Figure 2.3: Breadth of T cell responses to HCoV-NL63 protein. The numbers of SFU per million PBMCs **(A)** and stimulation indices **(B)** generated for pools of 10 peptides are shown for 10 HDs. Horizontal bars indicate the median. Pools that elicited the most potent responses are highlighted in red.

2.4.3. Expansion of memory T cells and cross-recognition of HCoV-NL63 and SARS-CoV-2 S protein peptide pools.

We cultured PBMCs with peptide pools from different viral proteins to determine whether we could detect memory $CD4^+$ T cell responses that were not seen when PBMCs were assayed directly after isolation. As shown in Figure 2.4, preculturing of PBMCs with the HCoV-NL63 S peptide pool caused an increase in the percentage of HD and CCP $CD4^+$ T cells that coexpressed either IFN-γ and IL-2 (Figure 2.4, A and B) or IFN-γ and TNF-α (Figure 2.4, C and D) when the cells were restimulated with the same peptide pool. Interestingly, a modest

but significant increase was also seen when cells from HDs were precultured and stimulated with SARS-CoV-2 S peptide pools, suggesting that memory responses to these peptides could be amplified in some HDs. HD9, the only individual who had a positive ELISPOT response to the SARS-CoV-2 peptide pool, also had the most robust memory T cell responses to both HCoV-NL63 and SARS-CoV-2 S peptide pools. We performed the preculture expansion assay to determine whether cross-recognition could potentially explain this observation. As shown in Figure 2.5, PBMCs cultured in the absence of antigen for 10 days did not produce responses to HCoV-NL63 or SARS-CoV-2 S proteins or to the SARS-CoV-2 N protein that were above background levels (Figure 2.5A, plots 1–4) following a 12-hour restimulation with each peptide pool. In contrast, following 10 days of culturing with HCoV-NL63 S protein peptides, a 12-hour restimulation with the same peptides induced coexpression of IFN-γ and IL-2 from 1.25% of $CD4^+$ T cells, a 9.6-fold increase over the response obtained when the cells were pre-cultured without peptide (Figure 2.5A, plot 6 vs. plot 2). Interestingly, when cells that were cultured with the HCoV-NL63 S peptide pool for 10 days were restimulated with SARS-CoV-2 S peptides, we detected coexpression of IFN-γ and IL-2 in 0.41% of $CD4^+$ T cells (Figure 2.5A, plot 8). This represents a 2.6-fold increase over cells that were precultured for 10 days in the absence of peptide and then stimulated with SARS-CoV-2 S peptides (Figure 2.5A, plot 4). Of note, we observed no increase in the percentage of cells that recognized SARS-CoV-2 N peptides following preculturing with the HCoV-NL63 S peptide pool (Figure 2.5A, plot 7 vs. plot 3), suggesting that the increase

in SARS-CoV-2 S peptide–reactive cells was not due to nonspecific stimulation. Thus, it is likely that there was $CD4^+$ T cell cross-recognition of S peptides from the 2 viruses. We observed similar 2.6- and 3-fold increases in antigen-responsive $CD4^+$ T cells when PBMCs precultured with SARS-CoV-2 S peptides for 10 days were restimulated with SARS-CoV-2 (Figure 2.5A, plot 16 vs. plot 4) and HCoV-NL63 (Figure 2.5A, plot 14 vs. plot 2) S peptide pools, respectively, which is further evidence of T cell cross-recognition in HD9.

We performed the same experiment with PBMCs from a CCP (CCP2). Preculturing of PBMCs with HCoV-NL63 S pep- tides resulted in 42.6- and 10.0-fold increases in the percentage of cells that responded to restimulation with HCoV-NL63 (Figure 2.5B, plot 22 vs. plot 18) and SARS-CoV-2 S peptides (Figure 2.5B, plot 24 vs. plot 20), respectively. Interestingly, while preculturing of the PBMCs with the SARS-CoV-2 S peptide resulted in a 76.8-fold increase in the percentage of cells that responded to restimulation with the SARS-CoV-2 S peptide pool (Figure 2.5B, plot 32 vs. plot 20), no such increase was seen in the percentage of $CD4^+$ T cells that responded to restimulation with HCoV-NL63 S peptides (Figure 2.5B, plot 30 vs. plot 18). Thus, the memory $CD4^+$ T cells that were amplified by the S peptides from the 2 viruses most likely had different T cell receptor repertoires with different cross-recognition capacities. We obtained similar cross-recognition results with PBMCs from another CCP, CCP3 (Figure 2.6) and in this case, preculturing of PBMCs with SARS-CoV-2 S peptides also resulted in cross-recognition of the NL63 S peptide pool.

We generally did not see amplification of HD $CD8^+$ T cell responses after preculturing with HCoV-NL63 S or SARS-CoV-2 S or N peptide pools (Figure 2.7). However, $CD8^+$ T cells coexpressing TNF-α and IFN-γ in response to SARS-CoV S and N peptide pools were amplified in CCP3 in the expansion assay, and there was again evidence of cross-recognition of HCoV-NL63 and SARS-CoV-2 S peptides, suggesting that this phenomenon was not limited to $CD4^+$ T cells (Figure 2.8).

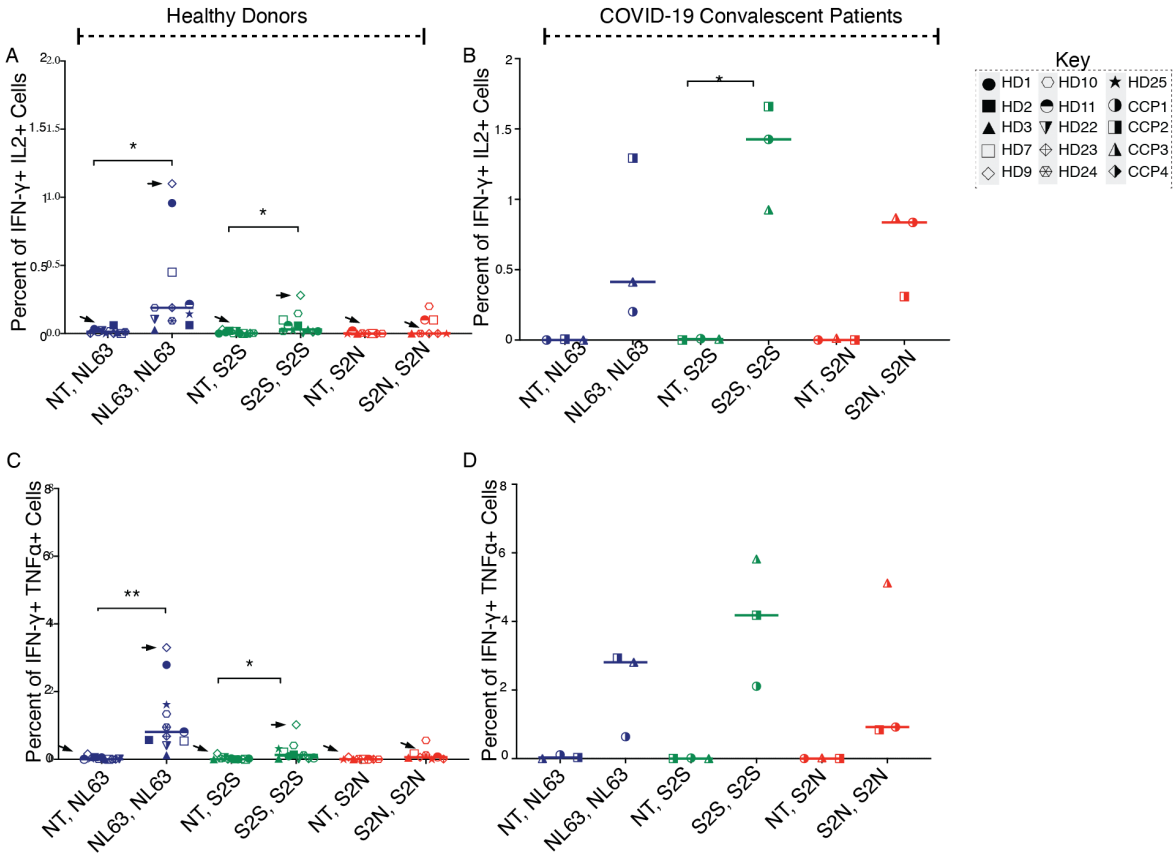

Figure 2.4: Expansion of antigen-specific CD4+ T cell responses. The percentages of cells that co-expressed either IL-2 or IFN-γ **(A and B)** or TNF- α **(C and D)** are shown

for cells from HDs (A and C) and CCPs (B and D) following pre-culturing for 10-12 days and stimulation for 12 hours with varied peptide pools (n=11 HDs; n=3 CCPs). In each panel, the peptide pool used for preculturing is shown first, followed by the peptide pool used in the 12-hour stimulation. *P = 0.0332 and **P = 0.0021, by 2-tailed, paired student's t test. Horizontal bars represent the median. NT, untreated; NL63, HCoV-NL63; S2N, SARS-CoV-2-N; S2S, SARS-CoV-2-S.

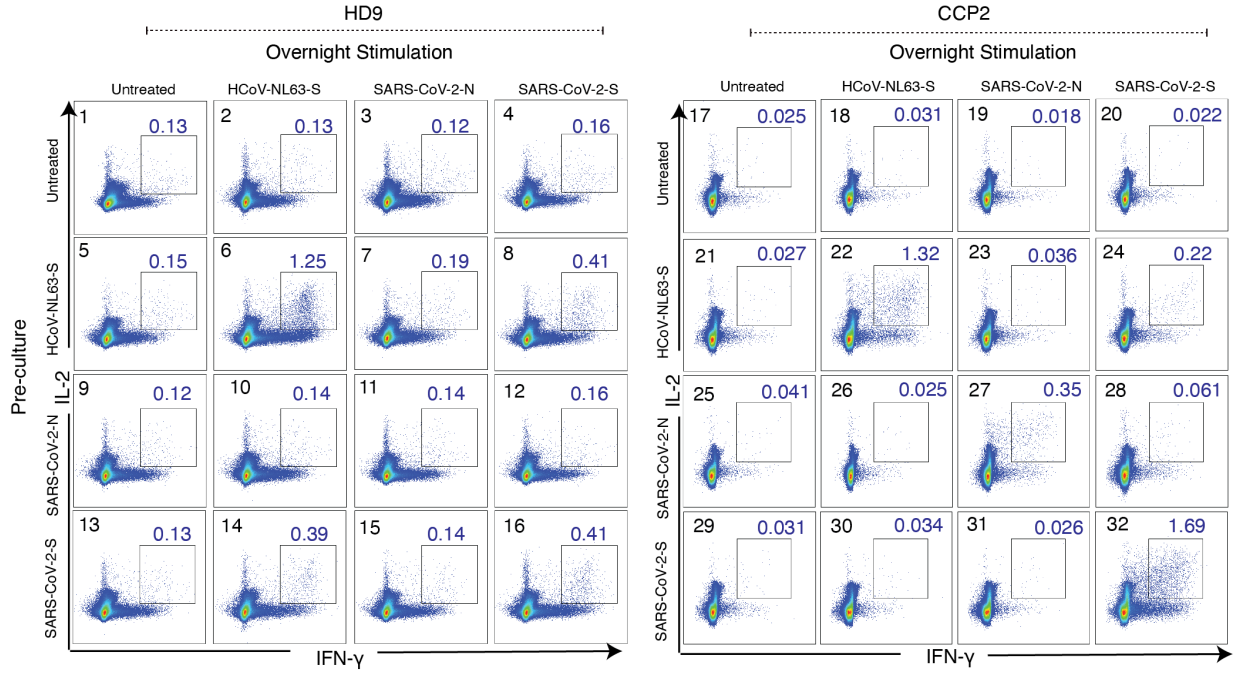

Figure 2.5: Cross-recognition of HCoV-NL63 and SARS-CoV-2 S protein peptide pools in HD9 and CCP2. PBMCs from HD9 (A) and CCP2 (B) were precultured with peptide pools (shown in rows) for 10–12 days and then stimulated for 12 hours with peptide pools (shown in columns). The percentage of cells that co-expressed IL-2 (y

axis) and IFN-γ (x axis) is shown above the gated box in the upper right corner of each plot.

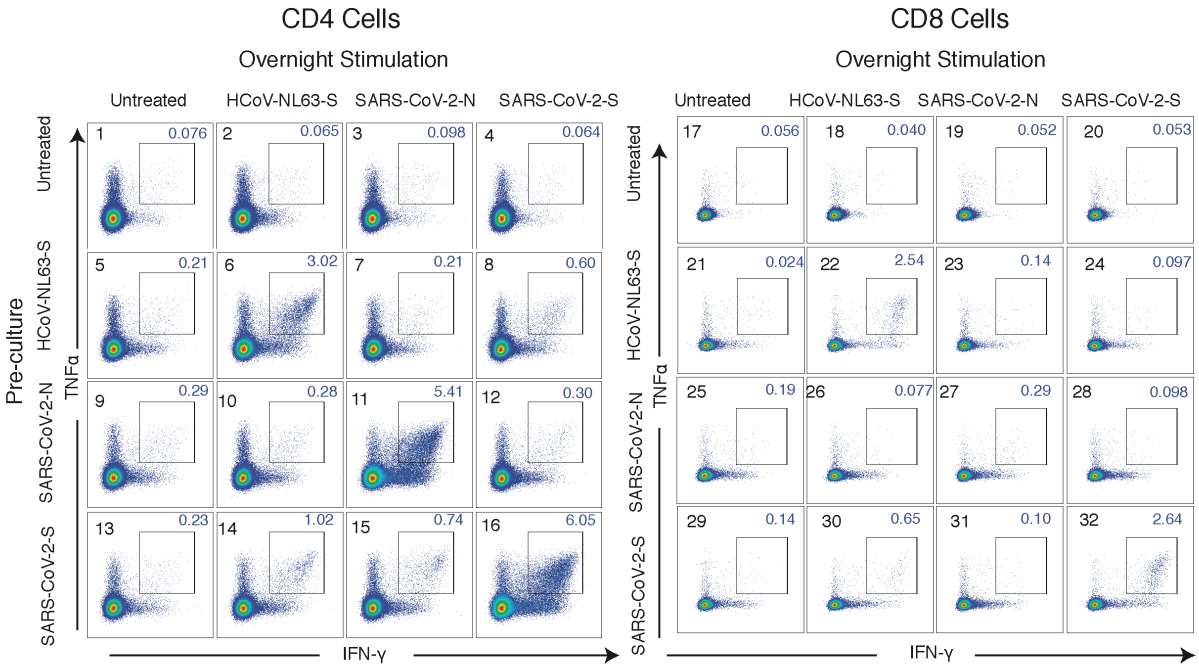

Figure 2.6: Expansion of antigen-specific memory CD4+ and CD8+ T cell responses from a COVID-19 convalescent patient (CCP3). PBMCs were pre-cultured with peptide pools for 12 days (shown in rows), and then stimulated for 12 hours with peptide pools shown in columns. The percentage of cells that co-express IFN-γ (x-axis) or TNFα (y-axis) are shown above the gated box in the upper right corner of each plot.

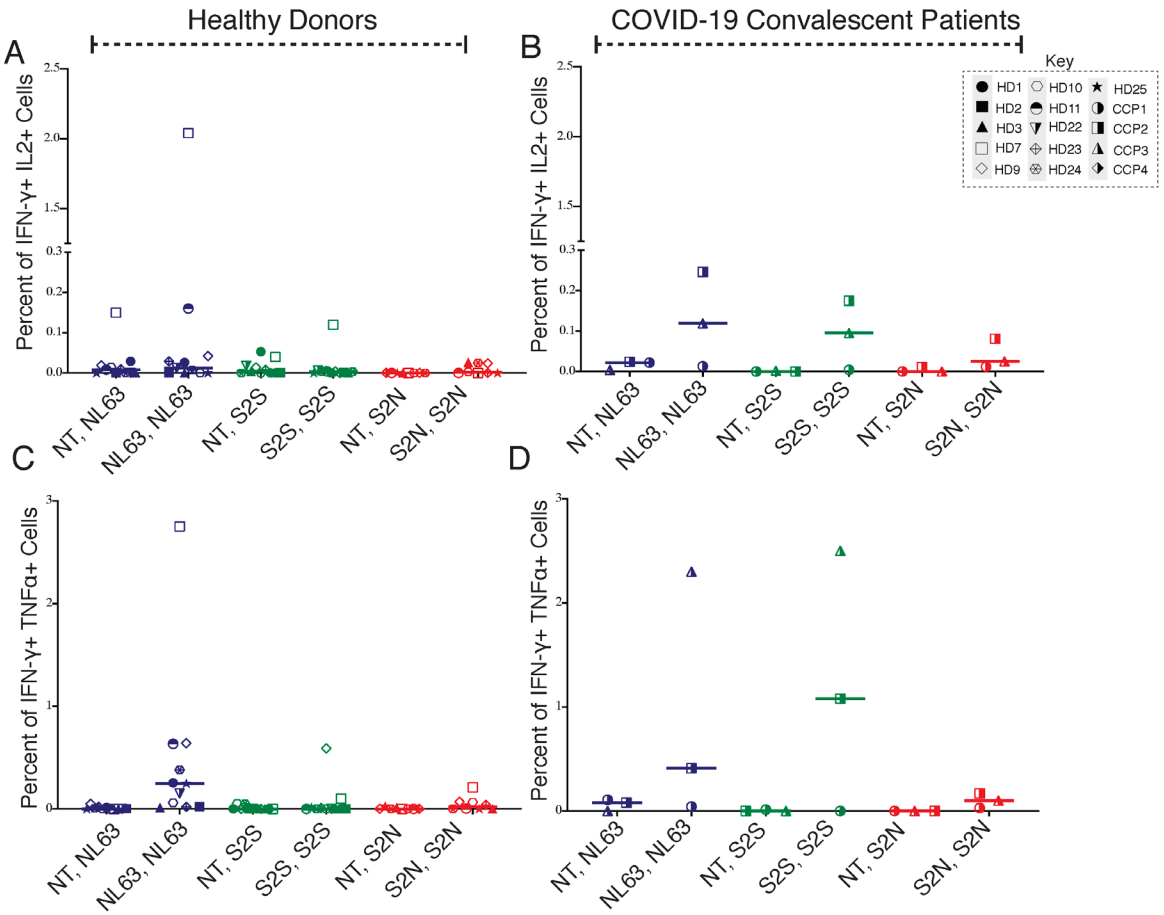

Figure 2.7: Expansion of antigen-specific memory CD8+ T cell responses. The percentage of cells that co-ex-press either IL-2 and IFN-γ **(A, B)** or TNFα and IFN-γ **(C, D)** are shown in cells from healthy donors **(A,C)** or COVID-19 convalescent patient T Cells **(B,D)** following pre-culture for 10 - 12 days and stimulation for 12 hours with peptide pools (n=11 for healthy donors, and n=3 for COVID-19 convalescent patients). In each figure, the peptide pool used to pre-culture is shown first, followed by the peptide pool

used in the 12-hour stimulation. Horizontal bars represent the median. NT = Untreated, NL63 = HCoV-NL63, S2N = SARS-CoV-2-N, S2S= SARS-CoV-2-S.

Table 2.1: HCoV-NL63 T cell-targeted peptides detected by ELISpot assay and HLA-binding predictions

HD ID (HLA alleles)	Peptide number	Amino acid number	Targeted peptide (predicted optimal epitope underlined)	Predicted HLA-restricting alleles	SFU/10^6 PBMCs
HD2 DRB1*01:01, 07:01, DRB4* 01:01, DQA1*02:01/DQB1*02:02 DQA1*01:01/DQB1*05:01 DPA1*01:03/DPB1*04:01	16[A]	91–107	VTNEIGLNASVTLKICK	DRB1*07:01, DRB1*01:01	26
	75	442–458	FEKLQCEHLQFGLQDGF	DRB4*01:01, DPA1*01:03/DPB1*04:01	48
	133	789–805	CATYVCNGNPRCKNLLK	DRB1*01:01, DRB1*07:01	30
	161	956–972	ARLNYVALQTDVLQENQ	DQA1*02:01/DQB1*02:02, DRB1*01:01	30
	166	986–1002	IVASFSSVNDAITQTAE	DQA1*02:01/DQB1*02:02, DRB1*07:01	26
HD4 DRB1*01:03, 13:05, DRB3* 02:02, DQA1*01:01/DQB1*05:01, DQA1*05:05/DQB1*03:01, DPA1*01:03/DPB1*02:01, DPA1*01:03/DPB1*04:01	134[B]	795–811	NGNPRCKNLLKQYTSAC	DRB3*02:02	28
	142	843–859	ANVTSFGDYNLSSVLPQ	DQA1*01:01/DQB1*05:01	36
	147	873–889	LEDLLFSKVVTSGLGTV	DRB1*01:03, DRB1*13:05	30
HD5 DRB1*07:01,11:01, DRB3*0202, DQA1*02:01/DQB1*03:03, DQA1*05:05/DQB1*03:01, DPA1*01:03/DPB1*04:01, DPA1*02:01/DPB1*13:01	16[A]	91–107	VTNEIGLNASVTLKICK	DRB3*02:02, DRB1*07:01	44
	132	783–799	TPIWDCATYVCNGNPR	DRB3*02:02, DQA1*02:01/DQB1*03:03	30
HD7 DRB1*04:04,11:01, DRB3*0202, DRB4*0103, DQA1*03:01/DQB1*03:02, DQA1*05:05/DQB1*03:01, DPA1*01:03/DPB1*06:01, DPA1*02:01/DPB1*14:01	17	97–113	LNASVTLKICKFSRNTT		64
	20	115–131	DFLSNASSSFDCIVNLL	DRB3*02:02, DQA1*05:05/DQB1*03:01	38
	150	891–907	VDYKSCTKGLSIADLAC	DRB1*11:01 DQA1*05:05/DQB1*03:01	68
HD9 DRB1*11:01,14:02, DRB3*0101, 0202, DQA1*05:03/DQB1*03:01, DQA1*05:05/DQB1*03:01, DPA1*01:03/DPB1*04:01, DPA1*01:03/DPB1*04:02	18	103–119	LKICKFSRNTTFDFLSN	DRB3*02:02	80
	62	364–380	TFVGILPPTVREIWAR		35
	71	418–434	ATFVDVLVNVSATNIQN		40
	132	783–799	TPIWDCATYVCNGNPR	DRB3*01:01	65
	141	837–853	SNAFSLANVTSFGDYNL	DRB3*02:02, DRB1*14:02	180
	146[C]	867–883	IAGRSALEDLLFSKVVT		108
	158	938–954	VLGGLTSAAAIPFSLAL	DQA1*05:03/DQB1*03:01	48
	160	950–966	FSLALQARLNYVALQTD	DRB1*14:02	45
	192	1139–1155	KNVKAWSGICVDGIYGY	DQA1*05:05/DQB1*03:01	70
	196	1163–1179	VLYSDNGVFRVTSRVMF		55
	205	1217–1233	VNKTLQEFAQNLPKYVK	DRB3*02:02, DRB1*14:02	50
HD10 DRB1*04:05,04:07, DRB4*0103, DQA1*03:03/DQB1*03:01, DQA1*03:03/DQB*03:02, DPA1*01:03/DPB1*03:01, DPA1*01:03/DPB1*04:01	141	837–853	SNAFSLANVTSFGDYNL	DRB1*04:07, DQA1*03:03/DQB1*03:01	38

[A]Peptide 16 partially overlaps with an HCoV-NL63 peptide that was found to be homologous to a SARS-CoV-2 peptide targeted by unexposed HDs (S 96-110) (16). [B]Peptide 134 partially overlaps with an HCoV-NL63 peptide that was found to be homologous to a SARS-CoV-2 peptide targeted by unexposed HDs (S 802-816) (16). [C]Peptide 146 partially overlaps with an HCoV-NL63 peptide that was found to be homologous to a SARS-CoV-2 peptide targeted by unexposed HDs (S 861-880) (16).

2.5 Discussion

In this study, we characterized the frequency of circulating common cold coronavirus–specific $CD4^+$ T cells in COVID-19–negative individuals. We show that many HDs who had not had upper respiratory syndromes in the past few months had a significant percentage of T cells that targeted the S protein of 3 common cold coronaviruses. The response to the HCoV-NL63 S protein appeared to be broad, and we identified 22 targeted peptides in this protein.

Several studies have looked for the presence of SARS-CoV-2–specific T cells in HDs. Peng et al. found no responses to various peptide pools by ELISPOT assays in 15 HDs from the United Kingdom (13), and Zhu et al. did not detect any baseline ELISPOT responses to S protein peptides in 108 vaccine recipients in China (21). In contrast, using an ELISPOT assay, Sekine et al. found T cells specific for the S and M, but not N, proteins in HDs in Sweden who donated blood prior to the pandemic (7). Le Bert et al. detect-ed responses to the N and nonstructural proteins in at least 30% of HDs in Singapore, also with the ELISPOT assay (8). Using upregulation of Ox40 and CD137 to detect T cell responses in PBMCs collected prior to the pandemic, Grifoni et al. found that $CD4^+$ T cells from 40%–60% of donors in the United States reacted to SARS-CoV-2 peptides (6). Weiskopf et al. found that $CD4^+$ T cells from 2 of 10 HDs in the Netherlands upregulated CD69 and CD137 in response to SARS-CoV-2 peptides (11). Similarly, Braun et al. found that 35% of their HDs in Germany had $CD4^+$ T cell responses to SARS-CoV-2 spike protein peptide pools as determined by upregulation of 4-1BB and CD40L (9). The reason for this

baseline reactivity and the difference in the frequency of HDs with preexisting immunity to SARS-CoV-2 is unclear, but differences in expo-sure to common cold coronaviruses and potential cross-reactivity between T cells specific for these viruses and SARS-CoV-2 have been postulated as a possible explanation.

Although we did not analyze responses to the nonstructural proteins, we show here that most of our HDs did not have detectable responses to SARS-CoV-2 M, N, or S peptide pools by ELISPOT in spite of having detectable responses to 2 or 3 common cold coronaviruses. However, preculturing of cells with S peptide pools resulted in a modest but significant (P = 0.03) increase in the frequency of T cells that responded to these peptides, suggesting that memory T cell responses existed in some HDs. Although it is also possible that these were de novo responses, the expansion assay we used did not involve the stimulation of T cells with isolated DCs, and in prior experiments, we were unable to generate de novo responses to peptides (22).

Mateus et al. recently mapped out thirty-one SARS-CoV-2 S protein epitopes that were targeted by T cell lines from unexposed HDs (16). They showed that the homologous peptides in the S protein from the common cold coronaviruses were also recognized. These data suggest that this cross-recognition of viral epitopes by T cells can explain the preexisting immunity seen in some of their study participants. Notably, 28 of 31 of the homologous HCoV-NL63 S protein peptides identified by this approach were not targeted by $CD4^+$ T cells from the 6 HDs we tested, and this difference may partially explain the low number of individuals with preexisting SARS-CoV-2

immunity in our cohort. Interestingly, HD9, the only participant in our cohort who responded to SARS-CoV-2 peptide pools, had T cells that made a robust response to an HCoV-NL63 peptide (S 867-883) that overlaps significantly with a homologous HCoV-NL63 peptide (S 861-880) found to be targeted in 2 individuals in the Mateus et al. cohort (16). The S 861-880 peptide was found to have 53% homology to the SARS-CoV-2 S peptide (S 811-825) that elicited T cell responses in unexposed individuals.

A strength of our study is that we used an unbiased approach and examined the responses to overlapping peptides spanning the entire HCoV-NL63 S protein to determine targeted peptides. This approach is distinct from, and complimentary to, the approach used by Mateus et al., in which epitopes in the 4 common cold coronaviruses were detected by analyzing peptides that had homology to 142 SARS-CoV-2 epitopes (16). Our study is limited by the fact that we did not look at responses to HCoV-HKU1 protein and that we analyzed the responses to just the S protein. However, in studies in individuals with SARS (23) and COVID-19 ([6, 7, 9–16]), the S protein is quite immunodominant, so it is likely that the responses to the S protein peptides of the common cold coronaviruses we observed were representative of the responses to the entire viral proteome.

Another limitation is that, although we analyzed HD responses to SARS-CoV-2 S, M, and N peptide pools, we did not test for reactivity to SARS-CoV-2 nonstructural proteins. This is important, because some studies have shown responses to peptides from these antigens in unexposed donors ([6, 8, 16]). We

may also not have detected SARS-CoV-2–specific memory $CD4^+$ T cell responses in more HDs because we used a low concentration of IL-2 in our expansion assay in an effort to minimize nonspecific activation. Finally, we characterized cross-reactive T cell responses in just 1 unexposed HD, because HD9 was the only unexposed HD in our cohort with preexisting immunity to SARS-CoV-2. Although we screened this individual using 2 different SARS-CoV-2 antibody tests with stated sensitivities of 100% among hospitalized patients by 3 weeks after symptom onset (24, 25), negative findings do not definitively prove that this individual did not have asymptomatic infection. This is important, given the studies showing that seronegative exposed individuals can have SARS-specific T cell responses (7, 26), although the cross-reactivity we describe here may also explain those results.

We believe our data are important, because we interpreted the frequency of circulating SARS-CoV-2–specific effector T cells in HDs in the context of the frequency of HCoV-specific effector T cells. Furthermore, we show directly that in HD9, cross-recognition of SARS-CoV-2 peptides by HCoV-NL63–specific $CD4^+$ T cells could occur, and this can potentially explain previously described reports of preexisting immunity to SARS-CoV-2 in unexposed individuals, and is consistent with the results of Mateus et al. (16). Further studies in larger cohorts will be needed to determine how common these cross-reactive responses are. It will also be important to determine whether these responses lead to more rapid control of viral replication, thus conferring

protection, or whether they contribute to inflammation or suboptimal priming of SARS-CoV-2–naive T cells and lead to poor outcomes.

2.6 References

1. Ogimi C, Kim YJ, Martin ET, Huh HJ, Chiu CH, Englund JA. What's new with the old coronavirus- es? J Pediatric Infect Dis Soc. 2020;9(2):210–217.
2. Severance EG, et al. Development of a nucleo- capsid-based human coronavirus immunoassay and estimates of individuals exposed to coro- navirus in a U.S. metropolitan population. Clin Vaccine Immunol. 2008;15(12):1805–1810.
3. Kiyuka PK, et al. Human coronavirus NL63 molecular epidemiology and evolutionary patterns in rural coastal Kenya. J Infect Dis. 2018;217(11):1728–1739.
4. Galanti M, Shaman J. Direct observation of repeated infections with endemic coronavirus- es [published online July 7, 2020]. J Infect Dis. https://doi.org/10.1093/infdis/jiaa392.
5. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. Epi- demiol Infect. 1990;105(2):435–446.
6. Grifoni A, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with
7. COVID-19 disease and unexposed individuals. Cell. 2020;181(7):1489–1501.e15.

8. Sekine T, et al. Robust T cell immunity in convalescent individuals with asymptomat- ic or mild COVID-19 [preprint]. https://doi. org/10.1101/2020.06.29.174888. Posted on bioRxiv June 29, 2020.
9. Le Bert N, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls [published online July 15, 2020]. Nature. https://doi.org/10.1038/s41586- 020-2550-z.
10. Braun J, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19 [published online July 29, 2020]. Nature. https:// doi.org/10.1038/s41586-020-2598-9.
11. Meckiff BJ, et al. Single-cell transcrip- tomic analysis of SARS-CoV-2 reactive CD4+ T cells [preprint]. https://doi.org/10.1101/2020.06.12.148916. Posted on bioRxiv June 13, 2020.
12. Weiskopf D, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syn- drome. Sci Immunol. 2020;5(48):eabd2071.
13. Ni L, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID- 19 convalescent individuals. Immunity. 2020;52(6):971–977.e3.
14. Peng Y et al. Broad and strong memory CD4+ and CD8+ T cells induced by SARS-CoV-2 in UK con- valescent COVID-19 patients [preprint]. https:// doi.org/10.1101/2020.06.05.134551. Posted on bioRxiv June 8, 2020.
15. Neidleman J, et al. SARS-CoV-2-specific T cells exhibit phenotypic features of robust helper function, lack of terminal differentiation, and high

proliferative potential [published online August 9, 2020]. Cell Rep Med. https://doi. org/10.1016/j.xcrm.2020.100081.

16. Giménez E, et al. SARS-CoV-2-reactive interfer- on-γ-producing $CD8^+$ T cells in patients hospital- ized with coronavirus disease 2019 [published online Jun 24, 2020]. J Med Virol. https://doi. org/10.1002/jmv.26213.
17. Mateus J, et al. Selective and cross-reactive SARS- CoV-2 T cell epitopes in unexposed humans [published online August 4, 2020]. Science. https://doi.org/10.1126/science.abd3871.
18. Kroemer M, et al. COVID-19 patients display distinct SARS-CoV-2 specific T- cell respons- es according to disease severity [published online August 24, 2020]. J Infect. https://doi. org/10.1016/j.jinf.2020.08.036.
19. Sattler A, et al. SARS-CoV-2 specific T-cell responses and correlations with COVID-19 patient predisposition [published online August 24, 2020]. J Clin Invest. https://doi.org/ 10.1172/JCI140965.
20. Rodda LB, et al. Functional SARS-CoV-2-specific immune memory persists after mild COVID-19 [preprint]. https://doi.org/10.1101/2020.08.11.20171843. Posted on medRxiv August 15, 2020.
21. Snyder TM, et al. Magnitude and dynamics of the T-cell response to SARS- CoV-2 infection at both individual and population levels [preprint]. https://doi.org/10.1101/2020.07.31.20165647. Posted on medRxiv August 4, 2020.

22. Zhu FC, et al. Safety, tolerability, and immuno- genicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet. 2020;395(10240):1845–1854.
23. Pohlmeyer CW, et al. Cross-reactive microbial peptides can modulate HIV-specific CD8+ T cell responses. PLoS ONE. 2018;13(2):e0192098.
24. Li CK, et al. T cell responses to whole SARS coronavirus in humans. J Immunol. 2008;181(8):5490–5500.
25. Conklin SE, et al. Evaluation of serological SARS- CoV-2 lateral flow assays for rapid point of care testing [preprint]. https://doi.org/10.1101/2020.07.31.20166041. Posted on medRxiv August 4, 2020.
26. Van Elslande J, et al. Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by 4 automated immunoassays and 3 ELISAs [published online July 31, 2020]. Clin Microbiol Infect. https://doi.org/10.1016/j.cmi.2020.07.038.
27. Gallais F, et al. Intrafamilial exposure to SARS- CoV-2 induces cellular immune response without seroconversion [preprint]. https://doi.org/10.1101/2020.06.21.20132449. Posted on medRxiv June 22, 2020.
28. Vita R, et al. The Immune Epitope Data- base (IEDB): 2018 update. Nucleic Acids Res. 2019;47(D1):D339–D343.

29. Kwaa AKR, Talana CAG, Blankson JN. Interferon alpha enhances NK cell function and the suppres- sive capacity of HIV-specific CD8+ T cells. J Virol. 2019;93(3):e01541-18.
30. Veenhuis RT, et al. Long-term remission despite clonal expansion of replication-competent HIV-1 isolates. JCI Insight. 2018;3(18):e122795.

Contributions and Acknowledgments:

This chapter was published in the Journal of Clinical Investigation (Woldemeskel BA, et al. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. *J Clin Invest*. 2020;130(12):6631–6638.) with Abena Kwaa as a co-first author. Other author contributions are listed on the publication.

3 Chapter 3: SARS-CoV-2 mRNA Vaccines induce broad CD4+ T cell responses recognize HCoV-NL63 and the variants of concern

3.1 Abstract

Recent studies have shown T cell cross-recognition of SARS-CoV-2 and common cold coronavirus spike proteins. However, the effect of SARS-CoV-2 vaccines on T cell responses to common cold coronaviruses (CCCs) remains unknown. In this study, we analyzed $CD4^+$ T cell responses to spike peptides from SARS-CoV-2 and 3 CCCs (HCoV- 229E, HCoV-NL63, and HCoV-OC43) before and after study participants received Pfizer-BioNTech (BNT162b2) or Moderna (mRNA-1273) mRNA-based COVID-19 vaccines. Vaccine recipients showed broad T cell responses to the SARS-CoV-2 spike protein, and we identified 23 distinct targeted peptides in 9 participants,

including 1 peptide that was targeted in 6 individuals. Only 4 of these 23 targeted peptides would potentially be affected by mutations in the UK (B.1.1.7) and South African (B.1.351) variants, and $CD4^+$ T cells from vaccine recipients recognized the 2 variant spike proteins as effectively as they recognized the spike protein from the ancestral virus. Interestingly, we observed a 3-fold increase in the $CD4^+$ T cell responses to HCoV-NL63 spike peptides after vaccination. Our results suggest that T cell responses elicited or enhanced by SARS-CoV-2 mRNA vaccines may be able to control SARS-CoV-2 variants and lead to cross-protection against some endemic coronaviruses.

3.2 Introduction

T cell cross-recognition of SARS-CoV-2 and common cold coronaviruses (CCCs) has recently been demonstrated (1–10). The Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) mRNA COVID-19 vaccines generate robust T cell responses to spike peptides (11, 12), and we hypothesized that this may also translate to enhanced responses to CCCs. Multiple evolving spike protein variants have been described, and recent studies have generally shown some degree of reduction in the ability of mRNA vaccine–elicited antibodies to neutralize B.1.351 and/or B.1.1.7 variants (13–22). In this study, we analyzed $CD4^+$ T cell responses to CCCs before and after study participants received mRNA vaccines. We identified peptides targeted by $CD4^+$ T cells and determined whether they would be affected by mutations present in the B.1.351 and B.1.1.7 variants. Our data further our understanding of the impact of T cell cross-

recognition of coronaviruses.

3.3 Methods

Participants

Blood samples were obtained from 30 healthy individuals working in health care and laboratory donors who had not test- ed positive for COVID-19. Twelve participants were female and 18 were male. Eleven participants were between 20 and 29 years of age, 7 were between 30 and 39 years of age, 7 were between 40 and 49 years of age, and 5 were between 50 and 59 years of age. All participants in Figure 3.1 had blood drawn 7–14 days after the second shot, whereas all participants included in the variant study had blood drawn 7–11 weeks after the second shot. Twenty-eight participants received the Pfizer-BioNTech vac- cine, and 2 received the Moderna vaccine. For all experiments, PBMCs were collected from whole blood after Ficoll-Paque PLUS gradient centrifugation (GE Healthcare Life Sciences). For some experiments, $CD8^+$ T cells were depleted using Miltenyi Biotec $CD8^+$ T Cell Positive Selection Kits. High-resolution class II typing was performed on PBMCs from 6 healthy donors at the Johns Hopkins Hospital Immuno- genetics Laboratory. The Immune Epitope Database (IEDB) and Analysis Resource (http://www.iedb.org) was queried for optimal epitope and HLA-binding predictions using the recommended parameters (15).

Peptides and ELISPOT assays.

Peptides for the spike protein of HCoV-NL63, HCoV-229E, HCoV-OC43, and SARS-Cov-2 as well as the nucleocapsid protein of SARS-CoV-2 were obtained from BEI Resources and reconstituted with DMSO at a concentration of 10 mg/ml. The HCoV-229E S protein peptide pool has 195 peptides consisting of 17 mer with 11 amino acid overlaps. The HCoV-NL63 S protein peptide pool has 226 peptides made up of 14 –17 mer with 11–13 amino acid overlaps. The HCoV-OC43 S protein peptide pool has 226 peptides made up of 17 or 18 mer with 11 amino acid over- laps. The SARS-CoV-2 peptides are 12 mer, 13 mer, or 17 mer, with 10 amino acid overlaps. The spike protein peptide pool consisted of 181 peptides, and the nucleocapsid protein peptide pool consisted of 59 peptides. All the peptides were combined into 1 pool for each viral protein. Pools of 10 peptides were made for the SARS-CoV-2 S protein. Stimulation with anti-CD3 antibody (Mabtech, 1 μg/mL) was used as a positive control for each study participant. IFN-γ ELISPOT assays were performed as previously described (5). Briefly, ELISPOT Pro and ELISPOT Plus kits with precoated plates were purchased from Mabtech. The wells were plated with unfractionated PBMCs or $CD8^+$ T cell–depleted PBMCs at 250,000 cells/well, and the cells were cultured for 20 hours with HCoV peptides at a concentration of 10 μg/mL. The plates were then processed according to the manufacturer's protocol and read by a blind- ed independent investigator using an automated reading system. Four replicates per pool were run for comparison of the different viral proteins. The replicate furthest from the median was not used. If 2 values were equally distant from the median, then

the higher value was discarded. Two replicates were run for the SARS-CoV-2 S protein pools that examined the breadth of the T cell responses. For epitope mapping, each individual peptide present in a pool was tested in duplicate wells. A peptide was only considered to be positive if both wells had values that were at least twice the average of the values of the untreated wells, the average stimulation index was above 3, and more than 20 SFU/106 cells were present.

SARS-CoV-2 variant assay

The S1 subunit of spike protein from ancestral SARS-CoV-2 (29) and the B.1.1.7 and B.1351 variants with polyhistidine tags at the C-terminus were purchased from Sino Biological and tested by ELISPOT assay at a concentration of 1 μg/mL

with a 20-hour incubation period. There were no significant responses made to these proteins by T cells from 4 unvaccinated healthy donors who had no known exposure to COVID-19. The mutations and deletions present in the variant proteins are shown in Figure 3.3C.

Expansion culture assay

PBMCs (10^7 cells) were cultured in R10 media with 10 U/mL IL-2 and 5 μg/mL peptides for 10–12 days as previously described (5). The media were not changed during this period. The cells were then washed and replated in fresh R10 with 10 U/mL IL-2 and rested 1 day before they were stimulated again with

5 μg/mL peptide with protein transport inhibitors (GolgiPlug, 1 μg/mL; GolgiStop, 0.7 μg/mL) and antibodies against CD28 and CD49d (all from BD Biosciences). After a 12-hour incubation, the cells were washed and stained with annexin V (BV-421, BD Biosciences, 563973) and antibodies against CD3 (APC-Cy-7, BioLegend, 300426) and CD4 (Per-CP-CY-5.5, BioLegend, 300530). The cells were then fixed, permeabilized, and stained intracellularly for TNF-α (PE-Cy-7, BD Biosciences, 557647) and IFN-γ (APC, BD Biosciences, 506510). Flow cytometry was performed on a BD FACS LSR Fortessa flow cytometer, and data were analyzed using FlowJo software, version 10. Data on a minimum of 100,000 events in the lymphocyte gate were collected and analyzed. *Statistics*. All statistical analyses were performed using GraphPad Prism 8.4.3 (GraphPad Software). For experiments requiring comparisons between 2 groups, a 2-tailed, paired Student's *t* test was used to determine significance. For experiments requiring multiple comparisons, a 1-way ANOVA with Greenhouse-Geisser correction was used. Dunnett's multiple-comparison test was used to determine differences between groups. A *P* value of less than 0.05 was considered statistically significant.

Study approval

The study was approved by the IRB of Johns Hopkins University. Written informed consent was obtained from all participants prior to their inclusion in the study.

3.4 Results and Discussion

The Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) mRNA vaccines elicit strong T cell responses to SARS-CoV-2 (11, 12). Given that recent studies have demonstrated cross-recognition of CCCs and SARS-CoV-2 by T cells (1–10), we asked whether COVID-19 vaccines would enhance T cell responses to the CCCs. We performed IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assays on PBMCs from individuals before and after vaccination to quantify the frequency of virus-specific T cells. As expected, the vaccines elicited strong T cell responses against SARS-CoV-2, with a median of 222 spot-forming units (SFU) per million cells responding to SARS-CoV-2 spike peptide pools after vaccination, compared with less than 3 SFU per million cells in the pre-vaccination samples (Figure 3.1, A and B). Furthermore, the vaccines elicited a significant increase in the response to HCoV-NL63, with an observed increase in the T cell response from a median of 28 SFU per million T cells before vaccination to a median of 93 SFU per million T cells after vaccination (Figure 3.1, A and B and Figure 3.2 A, B and E). We have previously shown that $CD4^+$ T cells were responsible for the majority of T cell responses generated by our peptide pools (5). Consistent with our prior study, we found that $CD8^+$ T cell depletion in PBMCs increased responses to all CCCs and SARS-CoV-2, (Figure 3.1, C and D and Figure 3.2 1C, D and F), suggesting that most of the T cell responses were due to CD4+ T cells. Specifically, we found that responses to HCoV-NL63 were enhanced, increasing from 36 SFU before vaccination to 113 SFU after vaccination.

Antigenic imprinting occurs when an initial response to a pathogen shapes the immune response to a subsequent infection by a related pathogen. This concept has been shown to play a role in $CD4^+$ T cell responses to influenza and other pathogens (23). Given the cross-reactive epitopes present in SARS-CoV-2 and CCC spike proteins (3, 6), we asked whether the ability of the vaccines to induce T cells responses to SARS-CoV-2 is affected by pre-existing T cell responses to CCCs. Interestingly, we found no correlation between T cell responses to CCCs prior to vaccination and T cell responses to SARS-CoV-2 after vaccination (Figure 3.1, E-G).

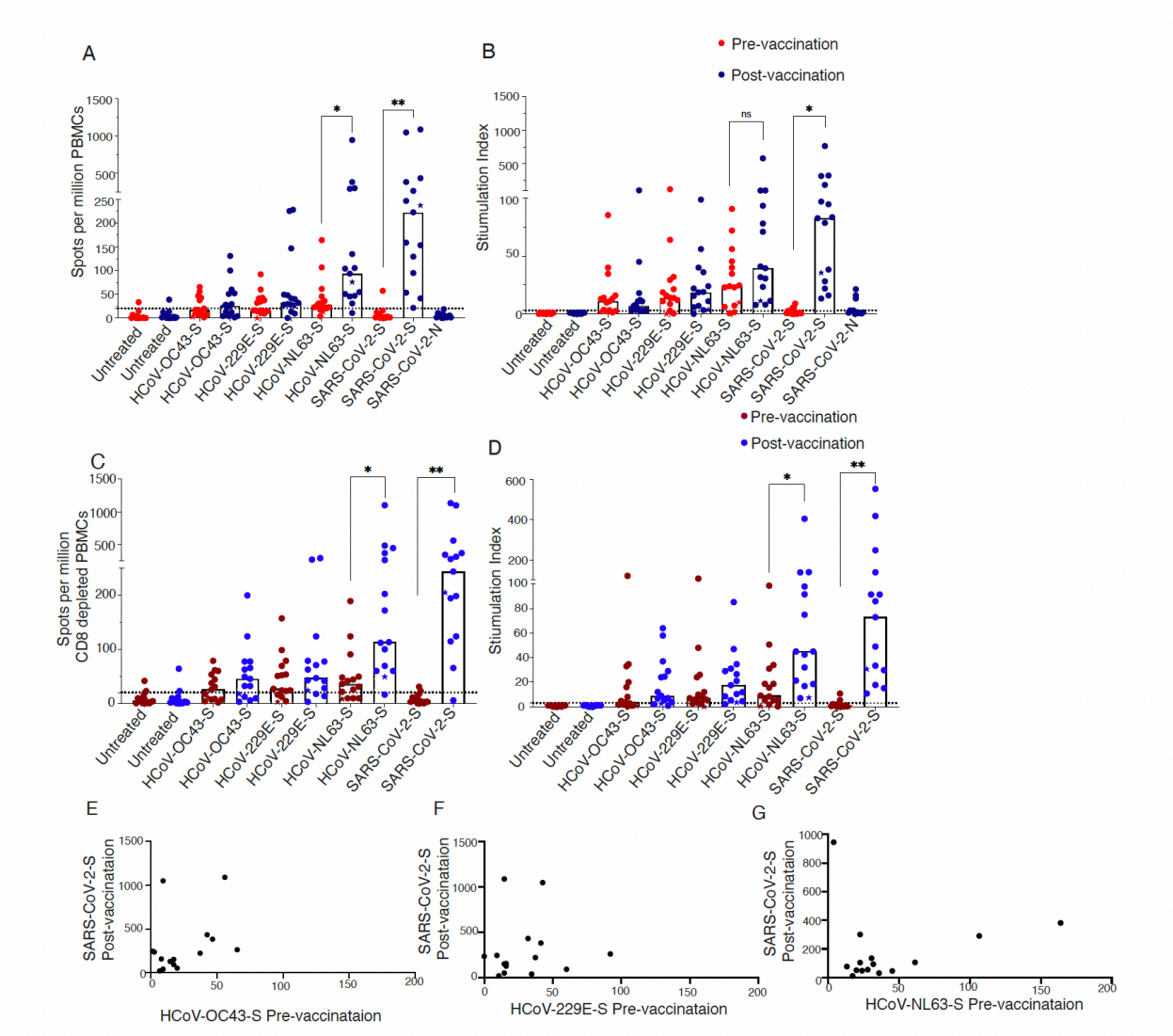

Figure 3.1: T cell IFN-γ responses to SARS-CoV-2 and CCCs. IFN-γ ELISpot was performed in samples obtained from individuals pre- and post-vaccination. The spot forming units (SFU) and stimulation indices of PBMCs **(A, B)** and CD8+ T cell depleted PBMCs **(C, D)** in response to HCoV-NL63, HCoV-229E, HCoV-OC43 and SARS-CoV-2 peptide pools are shown. Each data point represents the mean of 3 replicate values. Horizontal bars represent the median (n=15). Thedonor who received the Moderna (mRNA-1273) vaccine is indicated with a star symbol. *p = 0.0332 and **P = 0.0021, by

2-tailed, paired Student's t test. **(E-G)** Correlation between post-vaccination SARS-CoV-2 PBMC ELISpot responses and pre-vaccination responses to HCoV-OC43, HCoV-229E and HCoV-NL63 respectively. Pearson correlation test, r=0.065, 0.36 and 0.12

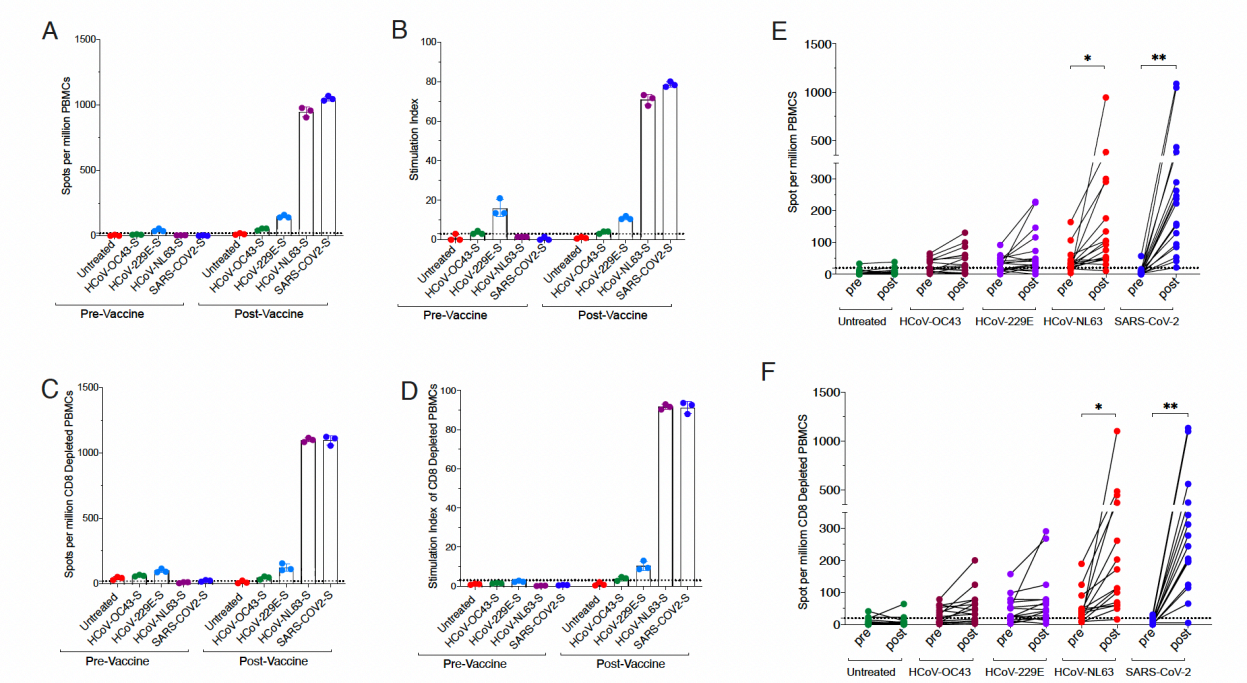

Figure 3.2: T cell IFN-γ responses to SARS-CoV-2 and CCCs (presented differently). A representative IFN-γ ELISpot from one donor (VR2) obtained pre-and post-vaccination (A-D). The spot formingunits (SFU) and stimulation indices of PBMCs (A, B) and CD8+ T cell depleted PBMCs (C, D) in response to HCoV-NL63, HCoV-229E, HCoV-OC43 and SARS-CoV-2 peptide pools are shown.IFN-γ ELISpot in PBMCs (E) and CD8 depleted PBMCs (F) in 15 donors obtained pre- and post-vaccination (replicate of Figure 3.1 to indicate change in T cell responses for each donor).Each data point represents the mean of 3 replicate values. Horizontal bars represent the median. *p = 0.0332 and **P = 0.0021, by 2-tailed, paired Student's t test. Horizontal bars represent the median. (E-G) Correlation between post-vaccination SARS-CoV-2 PBMC

ELISpot responses and pre-vaccination responses to HCoV-OC43, HCoV-229E and HCoV-NL63 respectively. Pearson correlation test, r=0.065, 0.36 and 0.12 respectively (n=15).

We next assessed whether vaccination enhanced T cell responses to HCoV-NL63 by generating antigen-specific T cell lines. Antigen-specific T cells before and after vaccination were expanded with peptide pools from HCoV-NL63 or SARS- COV-2 spike peptides for 10–12 days, and cytokine production was assessed following a 12-hour restimulation with the same peptide pools. As expected, we found that vaccination dramatically enhanced T cell responses to SARS-CoV-2 spike peptides, with a median of 4.2% of T cells coexpressing INF-γ and TNF-α after vaccination compared with 0.28% of T cells prior to vaccination (Figure 3.3, A and B). Interestingly, vaccination also dramatically enhanced T cell responses to HCoV-NL63 spike peptides, with a median of 2.7% of T cells coexpressing IFN-γ and TNF-α after vaccination compared with 0.4% before vaccination (Figure 3.3, A and B).

Thirteen of the 15 vaccine recipients studied (Figure 3.1) had preexisting T cell responses to HCoV-NL63, and we hypothesized that COVID-19 vaccination enhances responses to this virus as a result of an expansion of T cells that cross-recognize HCoV- NL63 and SARS-CoV-2 spike peptides. To test this hypothesis, we cultured cells with SARS-CoV2 spike peptide pools for 10–12 days and then restimulated the cells with peptide pools from a different virus (for example, cells expanded with SARS-CoV-2 spike peptides were then restimulated with SARS-CoV-2, HCoV- NL63, or HCoV-229E spike peptides). As shown in Figure 3.3C, cells

expanded with SARS-CoV-2 spike peptides for 10–12 days followed by restimulation for 12 hours with SARS-CoV-2 spike peptides had a dramatic increase in IFN-γ and TNF-α coexpression. Interestingly, these SARS-CoV-2–expanded T cells also responded to restimulation by HCoV-NL63 spike peptides, suggesting that vaccine-induced SARS-CoV-2–specific T cells also recognized HCoV-NL63 spike peptides in this study participant. Overall, in 9 vaccine recipients who were studied before and after vaccination, the percentage of SARS-CoV-2 spike peptide-specific $CD4^+$ T cells that cross-reacted with HCoV-NL63 spike peptides increased from 0.02% before vaccination to 0.28% after vaccination (Figure 3.3D). Further, following expansion with HCoV-NL63 peptides, the percentage of HCoV-NL63–specific $CD4^+$ T cells that cross-reacted with SARS-CoV-2 spike peptides increased from 0.005% before vaccination to 0.37% after vaccination (Figure 3.4).

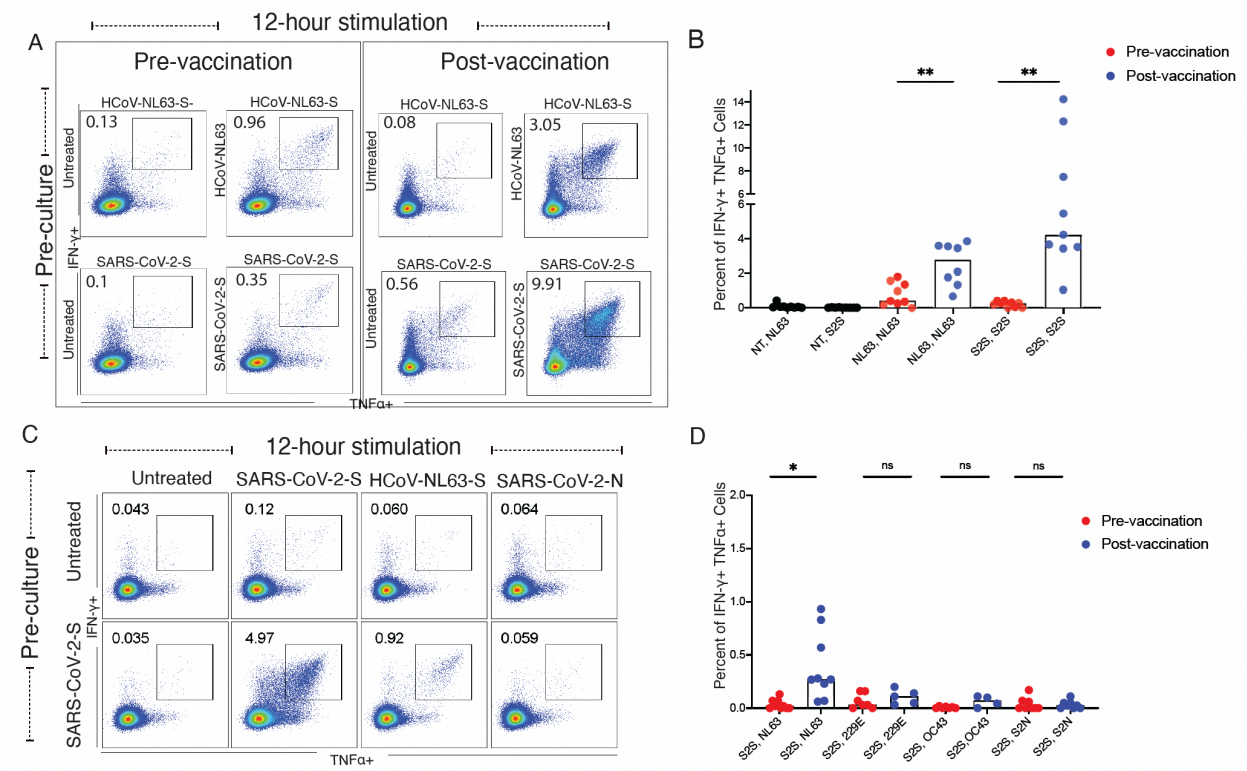

Figure 3.3: CD4+ T cell responses to SARS-CoV-2 and CCCs. CD4+ T cell responses after cells were cultured for 10-12 days, and then re-stimulated for 12-hours with HCoV-NL63 or SARS-CoV-2 spike peptide pools. TNF-α+ IFN-γ+ CD4+ T cells (shown in gated box) in response CCC or SARS-CoV-2 spike peptides are shown for a representative vaccine recipient (A) and for 9 vaccine recipients (B) pre and post-vaccination. (C-D) CD4+ T cell responses after cells were untreated or cultured for 10- 12 days with HCoV-NL63 or SARS-CoV-2 peptidepools (shown in rows), and then re-stimulated with different peptide pools for 12 hours (shown in columns) to analyze cross-reactive T-cell responses. Responses are shown for a vaccine recipient post-vaccination (C) and for 9 vaccine recipients pre and post-vaccination (D). In panels B and D, the peptide pool used for pre-culturing is shown first, followed by the peptide pool used in the 12-hour stimulation. NT

= not treated; NL63 = HCoV-NL63; 229E =HCoV-229E; OC43 = HCoV-OC43; S2N = SARS-CoV-2-N; S2S = SARS-CoV-2-S. *P = 0.0332

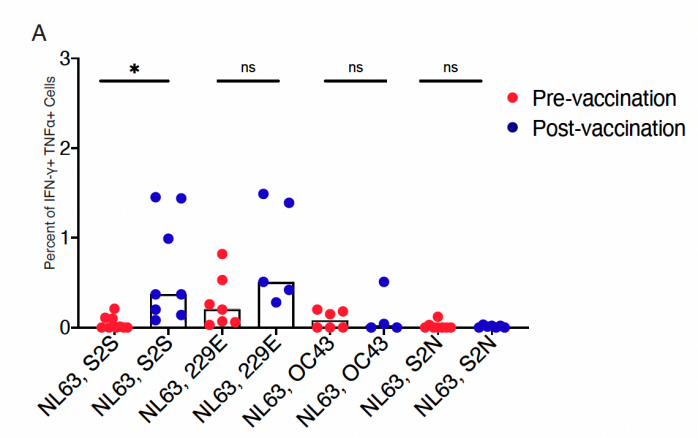

Figure 3.4: **HCoV-NL63 SpecificCD4+ T cell responses to re-stimulation by SARS-CoV-2 and CCC peptide pools.** Post-vaccination TNF-α+ IFN-γ+ CD4+ T cellresponses after cells were cultured for 10-12days with HCoV-NL63 peptide pools, and then re-stimulated for 12-hours with SARS-CoV-2 spike, HCoV-229E spike and HCoV-OC43 spike and SARS-CoV-2 nucleocapsid peptide pools to analyze cross-reactive T-cell responses. NT = not treated; NL63 = HCoV-NL63; 229E = HCoV-229E; OC43 =HCoV-OC43; S2N = SARS-CoV-2-N; S2S =SARS-CoV-2-S. *P = 0.0332 and **P = 0.0021, by 2-tailed, paired Student's t test.

Further studies are needed to determine why we observed a significant post-vaccination increase in the $CD4^+$ T cell response to HCoV-NL63 but not to HCoV-229E or HCoV-OC43 spike peptides. The percentage of sequence identity shared between the CCC spike proteins and the SARS-CoV-2 spike protein has been

estimated to be approximately 30%, with the β coronaviruses (HCoV-OC43 and HCoV-HKU1) having a slightly higher shared identity than the α coronaviruses (HCoV-NL63 and HCoV-229E, ref. 4). However, a recent study that analyzed antibodies against all 4 CCCs in plasma from convalescent COVID-19 patients revealed an association between HCoV-NL63 antibody responses and the development of highly neutralizing antibodies against SARS-CoV-2 (24), suggesting that HCoV-NL63 may have more epitopes in common with SARS-CoV-2 than the other CCCs.

We next mapped out individual spike peptides targeted by $CD4^+$ T cells. We performed IFN-γ ELISPOT assays with CD8-depleted PBMCs using sequential peptide pools consisting of 10 overlapping peptides. As shown in Figure 3.5, A and B, $CD4^+$ T cells recognized broad regions across SARS-CoV-2 spike in vaccine recipients, with pools containing peptides that covered amino acids 141–220, 351–430, 631–710, and 771–850 generating the most robust $CD4^+$ T cell responses. We then mapped specific peptides targeted in 9 vaccine recipients for whom we had a sufficient number of cells by repeating the ELISPOT assay with individual peptides from 3 of the 18 pools for each vaccine recipient. The optimal epitope and the predicted binding HLA allele were determined as previously described (5, 25). We identified 23 distinct targeted peptides (Table 3.1 and Table 3.2). One of these peptides (SKRSFIEDLLFNKVTLA, 813–829) was targeted in 6 of the 9 study participants. This epitope is present in a motif that is conserved in many coronaviruses (26), and the optimal epitope is predicted to bind to conserved HLA-DP alleles (Table 3.1).

Several spike variants have been described, and studies have shown that they are generally neutralized to a lesser extent by antibodies from mRNA vaccine recipients (13–22). However, it is unclear whether these variants also escape from T cells responses. This is a critical question, given the key role cellular immunity plays in controlling viral replication (27). Although $CD4^+$ T cell epitopes in convalescent COVID-19 patients have been characterized (3, 6, 28), little is known about epitopes targeted in vaccine recipients. In order to predict whether virus-specific T cells would still recognize and B.1351 variants, we determined whether mutations present in these variants were located in any of the targeted peptides we identified. Only 3 mutations (Y144 deletion, D614G, P681H) were present in any of the 23 targeted peptides, suggesting that these variants would be effectively recognized by the majority of vaccine-generated $CD4^+$ T cells. We tested this hypothesis by comparing $CD4^+$ T cell recognition of the S1 subunit of spike proteins from the ancestral virus with those from the B.1.1.7 and B.1351 variants. We found that the responses to the spike S1 subunits were lower than the responses to the spike peptide pools. This could be partly due to the proteins not being efficiently processed into peptides in the ELISPOT assay and the fact that S2 subunit epitopes were not present. We detected no significant difference in T cell responses to the S1 subunits from the ancestral virus or from the B.1.1.7 and B.1351 variants (Figure 3.5, D and E). This finding also held true for the 3 participants who were found to have targeted peptides that would be affected by the variant mutations. Overall, our data suggest that the mRNA vaccines may provide protection not just against SARS-CoV-2, but perhaps some CCCs as well.

Our data also suggest that vaccine-elicited $CD4^+$ T cells should effectively recognize some of the common SARS-CoV-2 variants and provide protection from severe disease even if the neutralizing antibodies are no longer effective.

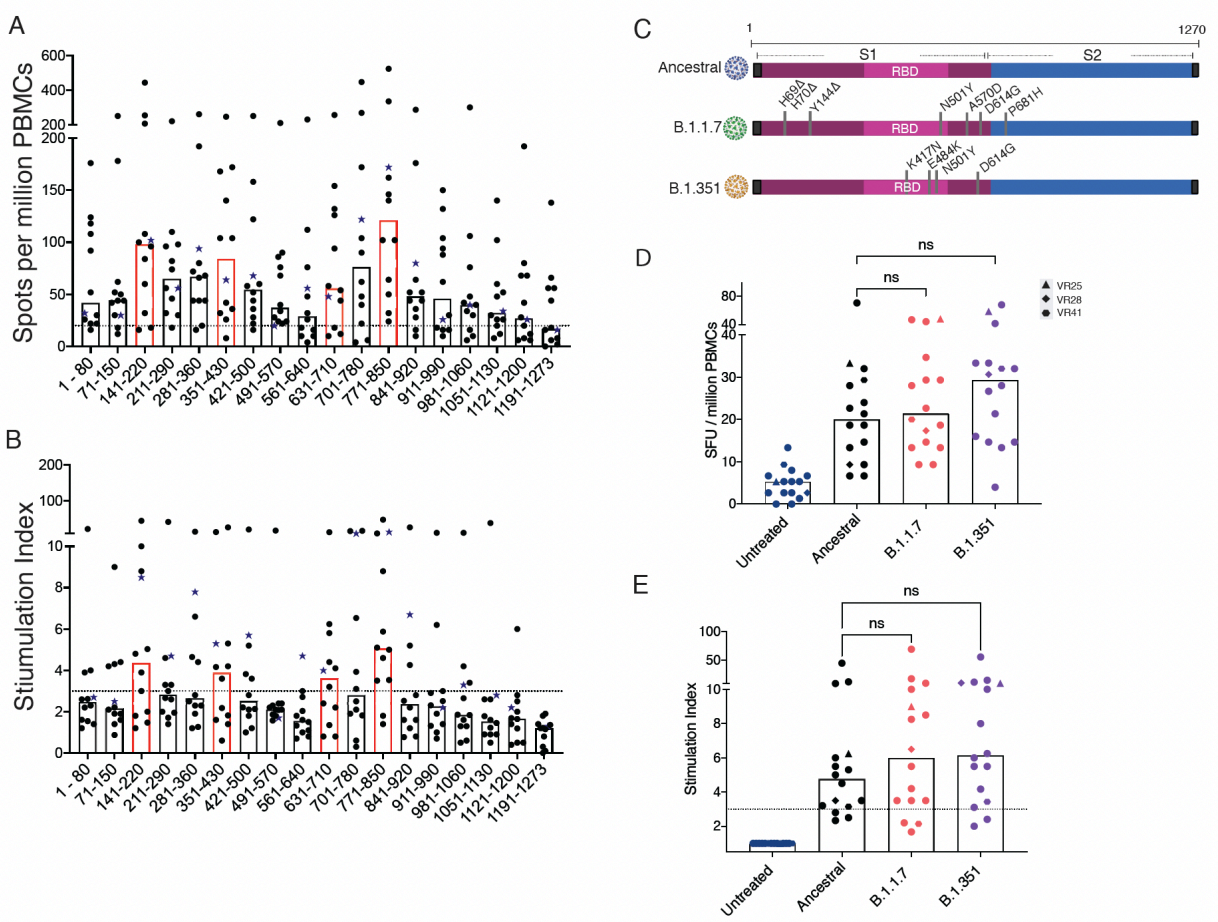

Figure 3.5: Breadth of T cell responses to SARS-CoV-2 spike protein and responses to spike variants. The numbers of SFU per million CD8+ T cell-depleted PBMCs **(A)** and stimulation indices **(B)** generated for pools of 10 peptides are shown for 12 vaccine recipients. The donor who received the Moderna (mRNA-1273) vaccine is indicated with a star symbol. Horizontal barsindicate the median. Pools that elicited the most potent responses are highlighted in red. T cell responses to S1 subunits from ancestral SARS-CoV-2 or B.1.351 and B.1.1.7 variant spike proteins **(C)** were measured. The numbers of SFU per million CD8+ T cell-depleted PBMCs **(D)** and stimulation indices **(E)** generated

are shown for 17 vaccine recipients. Horizontal bars represent the median. Statistical comparisons were performed using 1-way ANOVA with Geisser-Greenhouse correction and Dunnett's multiple-comparison test.

Table 3.1: Peptides targeted by vaccine recipients' CD4+ T cells

Peptide no.	Amino acid no.	Sequence	Responding VR	Predicted restricted HLA allele
2	8–24	LPLVSSQCVNLTTRTQL	VR32	DRB4*01:01
6	36–52	VYYPDKVFRSSVLHSTQ	VR32	DPA1*01:03/DPB1*04:01
13	85–101	PFNDGVYFASTEKSNII	VR28	DRB3*02:02
15	99–115	NIIRGWIFGTTLDSKTQ	VR28	DPA1*01:03/DPB1*06:01
18	120–136	VNNATNVVIKVCEFQFC	VR28	DRB3*02:02
20	134–150	QFCNDPFLGV**Y**YHKNNK	VR28	DRB1*11:01
21	141–157	LGV**Y**YHKNNKSWMESEF	VR41	DRB3*02:02
24	162–178	SANNCTFEYVSQPFLMD	VR32	DPA1*01:03/DPB1*04:01
28	190–206	REFVFKNIDGYFKIYSK	VR21, VR32	DQA1*01:01/DQB1*05:01 DRB5*01:01
51	351–367	YAWNRKRISNCVADYSV	VR25, VR28	DRB3*02:02, DRB4*01:03
54	372–388	ASFSTFKCYGVSPTKLN	VR20, VR28	HLA-DRB1*15:02 DPA1*01:03/DPB1*06:01
56	386–402	KLNDLCFTNVYADSFVI	VR28	DPA1*02:01/DPB1*14:01
58	400–416	FVIRGDEVRQIAPGQTG	VR28	DQA1*05:05/DQB1*03:01
64	442–458	DSKVGGNYNYLYRLFRK	VR14	DRB1*11:01
88	610–626	VLYQ**D**VNCTEVPVAIHA	VR25	DRB1*13:01
94	652–668	GAEHVNNSYECDIPIGA	VR25	DQA1*02:01/DQB1*02:02
98	680–696	S**P**RRARSVASQSIIAYT	VR41	DQA1*01:03/DQB1*06:03
103	715–731	PTNFTISVTTEILPVSM	VR32	DRB1*07:01
105	729–745	VSMTKTSVDCTMYICGD	VR20, VR32, VR41	DQA1*02:01/DQB1*02:02 DQA1*02:01/DQB1*03:03
108	750–766	SNLLLQYGSFCTQLNRA	VR21, VR36, VR40	DRB1*15:01
117	813–829	SKRSFIEDLLFNKVTLA	VR14, VR20, VR21, VR25, VR36, VR40	DPA1*01:03/DPB1*04:01 DPA1*02:01/DPB1*01: 01DPA1*02:02/DPB1*02:01
132	918–934	ENQKLIANQFNSAIGKI	VR28	DRB3*02:02
138	960–976	NTLVKQLSSNFGAISSV	VR28	DRB3*02:02

Amino acids in bold red font are present in B.1.1.7 and/or B.1.351 variants. VR, vaccine recipients.

Table 3.2: Complete list of peptides targeted by vaccine recipients' CD4+ T cells

VR	#	Amin oacid #	Targeted peptide	Predicted HLA-restricting alleles	SI
VR14 DRB1*11:01 DRB3* 02:02 DQA1*05:05/DQB1*03:01 DPA1*01:03/DPB1*02:01 DPA1*01:03/DPB1*04:01					
	64	442-458	DSKVGGNYNYLYRLFRK	DRB1*11:01	3.7
	117	813-829	SKRSFIEDLLFNKVTLA	DPA1*01:03/DPB1*04:01	5.1
VR20 DRB1*07:01, 15:02 DRB4* 01:01 DRB5*01:02 DQA1*02:01/DQB1*02:02 DQA1*01:03/DQB1*06:01 DPA1*01:03/DPB1*04:01					
	54	372-388	ASFSTFKCYGVSPTKLN	DRB1*15:02	
	105	729-745	VSMTKTSVDCTMYICGD	DQA1*02:01/DQB1*02:02	
	117	813-829	SKRSFIEDLLFNKVTLA	DPA1*01:03/DPB1*04:01	3.7
VR21 DRB1*03:01, 15:01 DRB3* 01:01 DRB5*01:01 DQA1*0501/DQB1*02:01 DQA1*01:02/DQB1*06:02 DPA1*02:01/DPB1*01:01 DPA1*02:06/DPB1*05:01					
	28	190-206	REFVFKNIDGYFKIYSK	DRB5*01:01	4
	108	750-766	SNLLLQYGSFCTQLNRA	DRB1*15:01	8.9
	117	813-829	SKRSFIEDLLFNKVTLA	DPA1*02:01/DPB1*01:01	8.0
VR25 DRB1*07:01, 13:01 DRB3* 02:02 DRB5*01:02 DQA1*02:01/DQB1*02:02 DQA1*01:03/DQB1*06:03 DPA1*01:03/DPB1*01:01 DPA1*02:02/DPB1*02:01					

	51	351-367	YAWNRKRISNCVADYSV	DRB3*02:02	4.1
	88	610-626	VLYQ**D**VNCTEVPVAIHA	DRB1*13:01	3.0
	94	652-668	GAEHVNNSYECDIPIGA	DQA1*02:01/DQB1*02:02	3.2
	98	680-696	S**P**RRARSVASQSIIAYT	DQA1*01:03/DQB1*06:03	4.1

	117	813-829	SKRSFIEDLLFNKVTLA	DPA1*02:02/DPB1*02:01	4.8
VR	Peptide	Amino acid	Targeted peptide	HLA alleles	SI
VR28 DRB1*04:04,11:01, DRB3*0202, DRB4*0103, DQA1*03:01/DQB1*03:02, DQA1*05:05/DQB1*03:01, DPA1*01:03/DPB1*06:01, DPA1*02:01/DPB1*14:01					
	13	85-101	PFNDGVYFASTEKSNII	DRB3*02:02	7.5
	15	99-115	NIIRGWIFGTTLDSKTQ	DPA1*01:03/ DPB1*06:01	14.5
	18	120-136	VNNATNVVIKVCEFQFC	DRB3*02:02	7.5
	20	134-150	QFCNDPFLGVYYHKNNK	DRB1*11:01	10.5
	51	351-367	YAWNRKRISNCVADYSV	DRB3*02:02, DRB4*01:03	18.5
	54	372-388	ASFSTFKCYGVSPTKLN	DPA1*01:03/DPB1*06:01	14.5
	56	386-402	KLNDLCFTNVYADSFVI	DPA1*02:01/DPB1*14:01	17.5
	58	400-416	FVIRGDEVRQIAPGQTG	DQA1*05:05/DQB1*03:01	14.5
	132	918-934	ENQKLIANQFNSAIGKI	DRB3*02:02	16
	138	960-976	NTLVKQLSSNFGAISSV	DRB3*02:02	22
VR32 DRB1*01:01, 07:01, DRB4* 01:01, DQA1*02:01/DQB1*02:02 DQA1*01:01/DQB1*05:01 DPA1*01:03/DPB1*04:01					
	2	8-24	LPLVSSQCVNLTTRTQL	DRB4*01:01	4.8

	6	36-52	VYYPDKVFRSSVLHSTQ	DPA1*01:03/DPB1*04:01	8.4
	24	162-178	SANNCTFEYVSQPFLMD	DPA1*01:03/DPB1*04:01	10.2
	28	190-206	REFVFKNIDGYFKIYSK	DQA1*01:01/DQB1*05:01 DRB5*01:01	8.8
	103	715-731	PTNFTISVTTEILPVSM	DRB1*07:01	8
	105	729-745	VSMTKTSVDCTMYICGD	DQA1*02:01/DQB1*02:02	13

VR36 DRB1*01:01, 15:01 DRB5*01:01 DQA1*01:01/DQB1*05:01 DQA1*01:03/DQB1*06:01 DPA1*01:03/DPB1*04:01					
	108	750-766	SNLLLQYGSFCTQLNRA	DRB1*15:01	3.2
	117	813-829	SKRSFIEDLLFNKVTLA	DPA1*01:03/DPB1*04:01	4.9
VR40 DRB1*03:01, 15:01 DRB3* 01:01 DRB5*01:0 1 DQA1*05:01/DQB1*02:01 DQA1*01:02/DQB1*06:02 DPA1*01:03/DPB1*04:01 DPA1*01:03/DPB1*06:01					
	108	750-766	SNLLLQYGSFCTQLNRA	HLA-DRB1*15:01	6.8
	117	813-829	SKRSFIEDLLFNKVTLA	DPA1*01:03/DPB1*04:01	4.7
VR41 DRB1*07:01,11: 01DRB3*0202 DQA1*02:01/DQB1*03:03 DQA1*05:05/DQB1*03:01 DPA1*01:03/DPB1*04:01 DPA1*02:01/DPB1*13:01					
	21	141-157	LGV**Y**YHKNNKSWMESEF	DRB3*02:02	4.8
	98	680-696	S**P**RRARSVASQSIIAYT	DQA1*02:01/DQB1*03: 03	3.6
	105	729-745	VSMTKTSVDCTMYICGD	DQA1*02:01/DQB1*03: 03	5.0

*Predicted optimal epitopes are underlined

SI= stimulation index

Amino acids in red font are present in B.1.1.7 and B.1.351 variants

3.5 References

1. Grifoni A, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020;181(7):1489–1501.
2. Le Bert N et al. SARS-CoV-2-specific T cell immu- nity in cases of COVID-19 and SARS, and unin- fected controls. *Nature*. 2020;584(7821):457–462.
3. Mateus J, et al. Selective and cross-reactive SARS- CoV-2 T cell epitopes in unexposed humans. *Science*. 2020;370(6512):89–94.
4. Braun J, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature*. 2020;587(7833):270–274.
5. Woldemeskel BA, et al. Healthy donor T cell respons- es to common cold coronaviruses and SARS-CoV-2. *J Clin Invest*. 2020;130(12):6631–6638.
6. Nelde A, et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat Immunol*. 2021;22(1):74–85.
7. Bacher P, et al. Low-avidity $CD4^+$ T cell responses to SARS-CoV-2 in unexposed individuals and humans with severe COVID-19. *Immunity.* 2020;53(6):1258–1271.
8. Richards KA, et al. Circulating CD4 T cells elicit- ed by endemic coronaviruses display vast dispari- ties in abundance and functional potential linked to both antigen specificity and age [published online February 8, 2021]. *J Infect Dis*. https://doi. org/10.1093/infdis/jiab076.
9. Sekine T, et al. Robust T Cell Immunity in Conva- lescent Individuals with

Asymptomatic or Mild COVID-19. *Cell*. 2020;183(1):158–168.

10. Tan HX, et al. Adaptive immunity to human coronaviruses is widespread but low in magnitude. *Clin Transl Immunology*. 2021;10(3):e1264.
11. Sahin U, et al. COVID-19 vaccine BNT162b1elicits human antibody and TH1 T cell responses.*Nature*. 2020;586(7830):594–599.
12. Jackson LA, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. *N Engl J Med.* 2020;383(20):1920–1931.
13. Wang Z, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants [pub- lished online February 10, 2021]. *Nature*. https:// doi.org/10.1038/s41586-021-03324-6.
14. Muik A, et al. Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. *Science*. 2021;371(6534):1152–1153.
15. Supasa P, et al. Reduced neutralization of SARS- CoV-2 B.1.1.7 variant by convalescent and vac- cine sera [published online February 18, 2021]. *Cell*. https://doi.org/10.1016/j.cell.2021.02.033.
16. Garcia-Beltran WF, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine- induced humoral immunity [published online March 12, 2021]. *Cell*. https://doi.org/10.1016/ j.cell.2021.03.013.
17. Edara VV, et al. Neutralizing antibodies against SARS-CoV-2 variants after infection and vacci- nation [published online March 19, 2021]. *JAMA*. https://doi.org/10.1001/jama.2021.4388.
18. Li Q, et al. SARS-CoV-2 501Y.V2 variants lack higher infectivity but do have immune escape [published online February 23, 2021]. *Cell*.

https://doi.org/10.1016/j.cell.2021.02.042.

19. Zhou D, et al. Evidence of escape of SARS-CoV-2 variant B.1.351 from natural and vaccine-induced sera [published online February 23, 2021]. *Cell*. https://doi.org/10.1016/j.cell.2021.02.037.

20. Collier DA, et al. Sensitivity of SARS-CoV-2 B.1.1.7 to mRNA vaccine-elicited antibodies [published online March 11, 2021]. *Nature*. https://doi.org/10.1038/s41586-021-03412-7.

21. Wang P, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7 [published online March 8, 2021]. *Nature*. https://doi.org/10.1038/ s41586-021-03398-2.

22. Chen RE, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies [published online March 4, 2021]. *Nat Med*. https://doi. org/10.1038/s41591-021-01294-w.

23. Nelson SA, Sant AJ. Imprinting and editing of the human CD4 T cell response to influenza virus. *Front Immunol*. 2019;10:932.

24. Morgenlander WR, et al. Antibody responses to endemic coronaviruses modulate COVID-19 convalescent plasma functionality. *J Clin Invest.* 2021;131(7):e146927.

25. Vita R, et al. The Immune Epitope Data- base (IEDB): 2018 update. *Nucleic Acids Res.* 2019;47(D1):D339–D343.

26. Robson B. COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and

analysis of a proposed achilles' heel conserved region to min- imize probability of escape mutations and drug resistance. *Comput Biol Med*. 2020;121:103749.

27. McMahan K, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature.* 2021;590(7847):630–634.
28. Tarke A, et al. Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. *Cell Rep Med*. 2021;2(2):100204.
29. Wu F, et al. A new coronavirus associated with human respiratory disease in China. *Nature.* 2020;579(7798):265–269.

Contributions and Acknowledgments:

This chapter was published in the Journal of Clinical Investigation (Woldemeskel BA, et al. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. *J Clin Invest.* 2021;131(10):e149335.) Data acquisition for flow cytometry assays were done by Caroline Garliss.

4 Chapter 4: CD4+ T-cells from COVID-19 mRNA vaccine recipients recognize a conserved epitope present in diverse coronaviruses

4.1 Abstract

Recent studies have shown that vaccinated individuals harbor T cells that can cross-recognize SARS-CoV-2 and endemic human common cold coronaviruses. However, it

is still unknown whether $CD4^+$ T cells from vaccinated individuals recognize peptides from bat coronaviruses that may have the potential of causing future pandemics. In this study, we identified a SARS-CoV-2 spike protein epitope ($S_{815-827}$) that is conserved in coronaviruses from different genera and subgenera, including SARS-CoV, MERS-CoV, multiple bat coronaviruses, and a feline coronavirus. Our results showed that $S_{815-827}$ was recognized by 42% of vaccinated participants in our study who received the Pfizer-BioNTech (BNT162b2) or Moderna (mRNA-1273) COVID-19 vaccines. Using T cell expansion and T cell receptor sequencing assays, we demonstrated that $S_{815-827}$-reactive $CD4^+$ T cells from the majority of responders cross-recognized homologous peptides from at least 6 other diverse coronaviruses. Our results support the hypothesis that the current mRNA vaccines elicit T cell responses that can cross-recognize bat coronaviruses and thus might induce some protection against potential zoonotic outbreaks. Furthermore, our data provide important insights that inform the development of T cell–based pan-coronavirus vaccine strategies.

4.2 Introduction

The COVID-19 pandemic has caused about 318 million infections and more than 5.5 million deaths since its emergence in Hubei province, China, in December 2019 (1). SARS-CoV-2, the virus that causes COVID-19, may have originated in bats (2–5). In the past 20 years, 2 additional highly pathogenic and transmissible coronavirus outbreaks with possible bat origins have occurred: SARS-CoV, which emerged in 2003, and MERS-CoV, which emerged in 2012 (3). Surveillance studies have shown that bats are reservoirs for SARS-related

and other genetically diverse coronaviruses (6). Zoonotic infections from bat-borne coronaviruses thus pose a major threat to humans, and the development of vaccines that can elicit robust, cross-reactive immunity across many coronaviruses is essential to protect against future pandemics (7).

Multiple vaccine candidates with high efficacy and immunogenicity against the original SARS-CoV-2 strain have recently been developed and administered worldwide (8–11). The COVID-19 mRNA vaccines generate strong T cell responses against the original SARS-CoV-2 strain (12) and variants of concern (13, 14). Robust T cell responses are associated with less severe COVID-19 infection (15), and T cell immunity has been shown to be protective against SARS-CoV-2, SARS-CoV, and MERS-CoV infections in animal models (16–18). In the past year, multiple groups have described the presence of cross-reactive T cells that can cross-recognize SARS-CoV-2 and endemic human common cold coronaviruses (HCoVs) (19–28). Additionally, the Pfizer-BioNTech (BNT162b2) and Moderna (mRNA-1273) vaccines have been shown to enhance HCoV-NL63–specific T cell responses after vaccination (14), suggesting enhanced vaccine-mediated immunity against this common cold coronavirus. T cell cross-reactivity is likely a result of sequence homology between SARS-CoV-2 and HCoVs. Computational studies have identified a highly conserved region within the fusion peptide domain of SARS-CoV-2 spike protein (29, 30). We have recently shown that some mRNA vaccine recipients target a peptide within this conserved region ($S_{813-829}$) (14). Furthermore, Loyal et al. have recently shown that more than 90% of COVID-19–vaccinated individuals in their cohort harbor T cells that recognize a peptide

located within the fusion peptide domain ($S_{816-830}$) and that these T cells are cross-reactive (31). Because $S_{813-829}$ is highly conserved in diverse coronaviruses, we hypothesized that vaccinated individuals could recognize this conserved epitope from bat coronaviruses not known to infect humans.

To test our hypothesis, we analyzed T cell responses against the conserved SARS-CoV-2 epitope $S_{815-827}$ in individuals we received two doses of COVID-19 mRNA vaccines. Our findings suggest that mRNA-vaccinated individuals have T cells responses that can cross-recognize multiple bat coronaviruses not currently known to infect humans. Our study will have implications for the development of T cell–oriented pan coronavirus vaccines that could protect against future zoonotic coronavirus outbreaks.

4.3 Methods

Study participants, biospecimens, and HLA haplotyping

COVID-19 convalescent patients (CCPs) are study participants who have tested positive for SARS-CoV-2 by nasal-swab PCR test in the past. All the CCPs in this study have received mRNA COVID-19 vaccination. The term VRs refers to participants who have never tested positive for SARS-CoV-2 and have received mRNA COVID-19 vaccination. All study participants worked in health care and / or laboratory settings. 33 participants received the Pfizer-BioNTech (BNT162b2) vaccine, and 5 participants received the Moderna (mRNA-1273) vaccine. Of total participants, 21 were ages 21-30, 7 were ages 31- 40, 8 were 41-50, and 2 were 51- 60. Blood was drawn and processed between June

- Aug 2021. Further details for participants who responded to the $S_{812-829}$ epitope are found in Table 4.3.

PBMCs were collected from whole blood after Ficoll-Paque PLUS gradient centrifugation (GE Healthcare Life Sciences), and CD8+ T cells were depleted using Miltenyi Biotech CD8+ T Cell Positive Selection Kits. High-resolution class II typing was performed by the Johns Hopkins Hospital Immunogenetics Laboratory. The Immune Epitope Database (IEDB) and Analysis Resource (http://www.iedb.org) was queried for optimal epitope and HLA-binding predictions using the recommended parameters (29).

4.3.2 Peptides and ELISPOT assays.

All peptide sequences were ordered from Genscript and reconstituted with DMSO at a concentration of 10 mg/mL. Anti-CD3 antibody (Mabtech, 1 μg/mL) was used as a positive control for each study participant. IFN-γ ELISPOT assays were performed as previously described (23). Briefly, ELISPOT Pro and ELISPOT Plus kits with precoated plates were purchased from Mabtech. The wells were plated with CD8+ T cell depleted PBMCs at 250,000 cells/well, and the cells were cultured for 20 hours with peptides at a concentration of 1 μg/mL. The plates were then processed according to the manufacturer's protocol and spots were read by a blinded independent investigator using an automated reading system that reported spot forming units (SFU) / well. Spot / million cells were calculated by multiplying spots / well by 4. Stimulation index (fold change over untreated control) for each donor was calculated by dividing SFU of peptide condition by SFU of untreated control. Four replicates were run for each condition, and the replicate

furthest from the median was not used. The mean of replicate values was used for plotting. A positive response was defined as a mean SFU ³ 20 and a mean of SI ³ 3.

T cell expansion culture assay.

10 – 20 million PBMCs were cultured in R10 media with 10 U/mL IL-2 and 5 μg/mL peptides for 10–12 days as previously described (23). The media were not changed during this period. The cells were then washed and replated in fresh R10 with 10 U/mL IL-2 and rested 1 day before they were stimulated again with 1 μg/mL peptide with protein transport inhibitors (GolgiPlug, 1 μg/mL; GolgiStop, 0.7 μg/mL) and antibodies against CD28 and CD49d (all from BD Biosciences). After a 12-hour incubation, the cells were washed and stained with annexin V (BV-421, BD Biosciences, 563973) and antibodies against CD3 (APC-Cy-7, BioLegend, 300426), CD4 (PerCP-CY-5.5, BioLegend, 300530) and CD8 (BV-605, BioLegend, 301040). The cells were then fixed, permeabilized, and stained intracellularly for TNF-α (PE-Cy-7, BD Biosciences, 557647) and IFN-γ (APC, BD Biosciences, 506510). Flow cytometry was performed on a BD FACS LSRFortessa flow cytometer, and data were analyzed using FlowJo software, version 10. Data on a minimum of 100,000 events in the lymphocyte gate were collected and analyzed.

***Identification of epitope-specific T cells*.**

Coronavirus peptides from SARS-CoV2, HCoV-NL63, MERS-CoV, NL63-related bat, 229E-related bat and Chaerephon bat coronavirus (listed in Table 4.1) were used to stimulate CD4+ T cells in the ViraFEST assay as described previously (18). Briefly, 2 × 10^6 PBMCs were plated in culture medium (IMDM, 5% human AB serum, 10 IU/ml IL-2,

50 µg/ mL gentamicin) with 1 µg/ml of peptide, a negative control HIV-1 Nef peptide pool (NIH AIDS Reagents), or without peptide. Each assay condition was performed in triplicate. On day 3 and 7 half the media was removed and replaced with fresh culture media. On day 10, cells were harvested and CD4+ T cells were isolated using the EasySep CD4+ T cell isolation kit (STEMCELL, 17952). DNA was extracted from cultured CD4+ T cells using the QIAmp Micro-DNA Kit according to the manufacturer's instructions (QIAGEN). TCR-Seq of DNA extracted from cultured CD4+ T cells was performed by the Johns Hopkins FEST and TCR Immunogenomics Core Facility (FTIC) using the Oncomine TCR Beta Short-Read Assay (Illumina Inc). Samples were pooled and sequenced on an Illumina iSeq 100 using unique dual indexes. Data preprocessing was performed to eliminate nonproductive TCR sequences and to align and trim the nucleotide sequences to obtain only the CDR3 region. Sequences not beginning with C or ending with F or W and having fewer than 7 amino acids in the CDR3 were eliminated. Resultant processed data files were uploaded to our publicly available MANAFEST analysis web app (http://www. stat-apps.onc.jhmi.edu/FEST/) to bioinformatically identify antigen specific T cell clonotypes. Clones were considered positive based on the following criteria: (a) significantly expanded in the culture of interest (in 2 of 3 replicate wells) compared with the reference culture (PBMCs cultured with 10 IU/ml IL-2 and HIV-1 Nef pool or media without peptide) at an FDR less than the specified threshold (< 0.05; default value) (c) having an odds ratio greater than 5 (default value), and (d) having a minimum of 0.1% frequency in two of three replicate wells. To identify cross reactive responses, we used statistical criteria established previously (30).

Statistics.

All statistical analyses were performed using GraphPad Prism 9.2.0 (GraphPad Software). Comparisons between two groups were done with Mann-Whitney test (if un-paired) and Wilcoxon matched-pairs signed rank test (if paired). Comparisons between multiple groups were done using Friedman test with Dunn's multiple comparison. A P value of less than 0.05 was considered statistically significant.

Study approval.

The study was approved by the IRB of Johns Hopkins University. Written informed consent was obtained from all participants prior to their inclusion in the study.

4.4 Results

Cross-reactive T cells are likely a result of sequence homology between SARS-CoV-2 and endemic HCoVs (29, 30). The SARS-CoV-2 spike peptide $S_{815-827}$ is found within the fusion peptide domain of the SARS-CoV-2 spike and is highly conserved in alpha and betacoronaviruses (29) (Figure 4.1A and Table 4.1). Additionally, the SARS-CoV-2 $S_{815-827}$ peptide sequence is identical in some coronaviruses found in the *Sarbecovirus* subgenus (Table 4.2). In this study, we looked at T cell responses in vaccinated individuals to $S_{815-827}$ and to homologous peptides from coronaviruses isolated from diverse hosts, including humans, bats, and felines (Table 4.1). We previously identified the 17-mer peptide $S_{813-829}$ to be targeted by $CD4^+$ T cells in some COVID-19 mRNA vaccine recipients (14). In this study, we synthesized 15-mer and 13-mer truncated peptides and performed IFN-γ enzyme-linked immunosorbent spot (ELISpot) assays in 3 vaccine recipients in order to determine the minimal peptide recognized by reactive T cells.

We identified a 13mer sequence $S_{815-827}$ (RSFIEDLLFNKVT) to be comparably recognized (Figure 4.2) and we proceeded to use this peptide for further experiments.

We next asked whether $S_{815-827}$ is recognized by the majority of COVID-19–vaccinated individuals. To test this, we isolated $CD8^+$ T cell–depleted PBMCs from 38 individuals vaccinated with Pfizer-BioNTech (BNT162b2) or Moderna (mRNA-1273) vaccines and performed IFN-γ ELISpot assays. All 38 individuals tested positive for antibodies to all 4 HCoVs by commercial ELISA kits, indicating prior exposure to these viruses. We found that 16 out of 38 (42%) of our donors (termed hereafter as responders) had robust T cell responses to $S_{815-827}$ and were above our cutoff of spot-forming unit (SFU) greater than or equal to 20 and stimulation index (SI) greater than or equal to 3 (Figure 4.1, B and C). For 3 donors for whom we had cryopreserved prevaccination samples, we performed IFN-γ ELISpot assays to determine whether responses to $S_{815-827}$ existed prior to COVID-19 vaccinations. None of the donors tested had responses to $S_{815-827}$ prior to vaccinations (Figure 4.2, B and C), indicating that at least in these donors, responses to $S_{815-827}$ were induced or expanded by vaccination. All responders had the HLA allele DPA1*01:03, and most had the predicted combined HLA binding allele DPA1*01:03/ DPB1*04:01, suggesting that this might be a restricting allele for $S_{815-827}$ (Table 4.3). We have previously shown that lymphoblastoid cell lines with DPA1*01:03/DPB1*04:01 are capable of presenting the related peptide $S_{813-829}$ (19).

Given that $S_{815-827}$ is a highly conserved epitope, we hypothesized that COVID-19–vaccinated individuals will have T cells that recognize homologous peptides from diverse coronaviruses with zoonotic potential. To test this hypothesis, we isolated $CD8^+$ T cell–depleted PBMCs from individuals who responded to $S_{815-827}$ and performed an IFN-γ ELISpot assay using homologous pep- tides from 9 coronaviruses, including HCoVs, MERS-CoV, bat coronaviruses, and a feline coronavirus (listed in Table 4.1). We found that all donors recognized at least 1 other coronavirus peptide, and 8 out of 15 donors recognized peptides from at least 6 out of the 9 other coronaviruses tested (Figure 4.1D). The coronaviruses most robustly recognized were common cold coronaviruses (HCoV-NL63, HCoV-HKU1), 229E-related bat coronavirus, and feline UU23 coronavirus (Figure 4.1, E and F).

Previous studies have shown that HCoV and SARS-CoV-2 cross-reactive $CD4^+$ T cells have lower functional avidity than SARS-CoV-2 monoreactive T cells (19, 26). Given that there could be functional avidity differences in T cells responding to $S_{815-827}$ and corresponding homologous peptides, we performed a peptide titration in 3 donors using the IFN-y ELISpot assay. Overall, we did not observe major differences in functional avidity to $S_{815-827}$ and homologous coronavirus peptides (Figure 4.2, D–F).

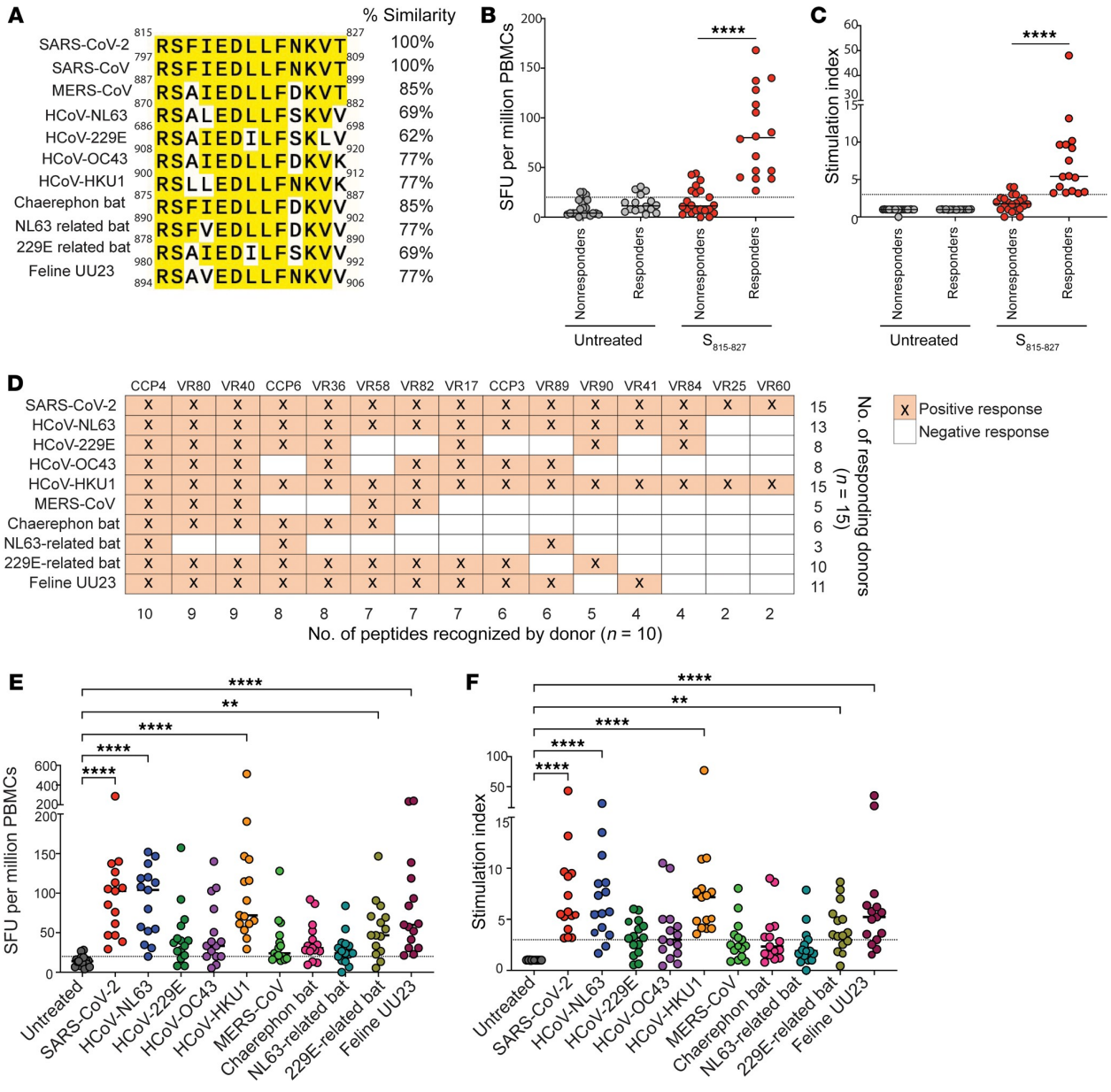

Figure 4.1: Some individuals vaccinated with COVID-19 mRNA vaccines have CD4+ T cells that recognize the conserved SARS-CoV-2 epitope $S_{815-827}$ and homologous peptides from diverse coronaviruses. Sequence alignment for coronavirus peptides used in this study are shown, highlighted are amino acid residues that are identical to $S_{815-827}$ (A). CD8+ T cell depleted PBMCs were isolated from 38

vaccinated individuals, and an IFN-g ELISpot assay was done in triplicate with S815-827 or untreated control. Mean of replicates was used to plot spot forming units (SFU) (B) and stimulation index (SI) (C). Responders (n=16) and non-responders (n=22) were determined based on the cut-off SFU ³ 20 and SI ³ 3. $S_{815\text{-}827}$ responders (n=15) were further assessed for CD4+ T cell responses to homologous coronavirus peptides with IFN-g ELISpot (D-F). Positive CD4+ T cell responses based on our cut-off for each individual donor and corresponding peptide are shown in orange (D). SFU and SI for donors are also shown (E, F respectively). Mann-Whitney test (B-C) and Friedman test with Dunn's multiple comparison (E-F) were used for statistical comparisons. P-values below 0.05 were considered statistically significant. **P = 0.0021, ****P< 0.0001

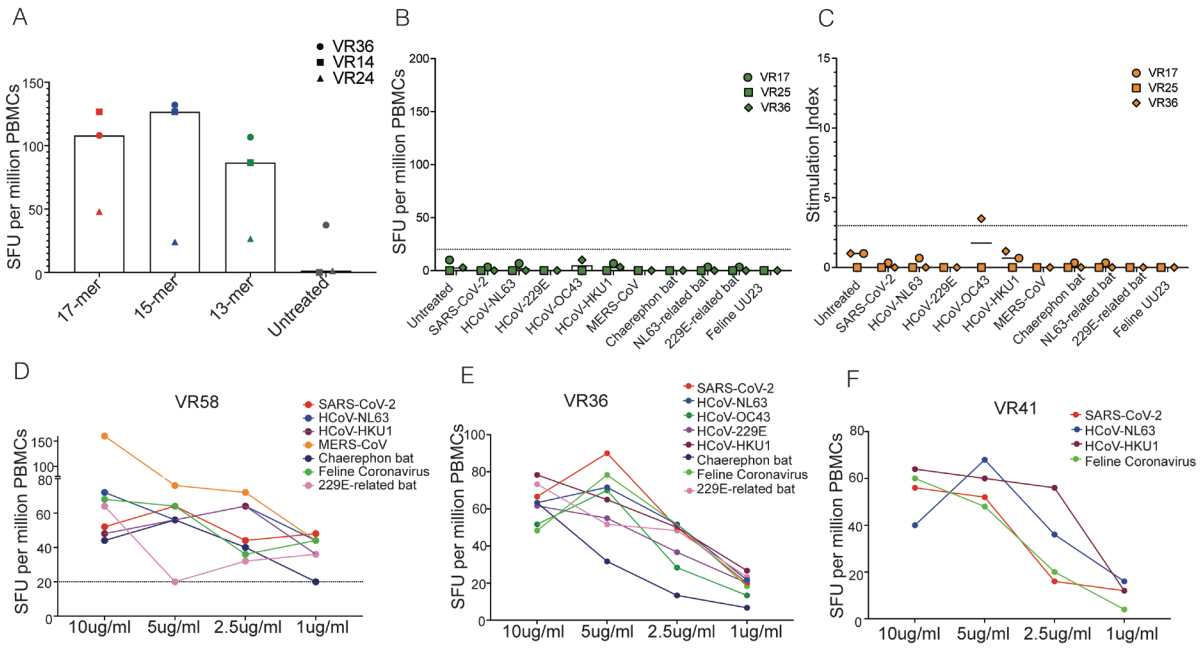

Figure 4.2: $S_{815-827}$ reactive T cells are not detected in pre-vaccination samples, and have similar functional avidity to T cells reactive to homologous peptides. The 13-mer peptide was determined by isolating PBMCs from 3 donors previously shown to recognize a 17-mer peptide $S_{813-829}$ (14). 15-mer and 13-mer truncated peptides were synthesized and an IFN-γ ELISpot assay was performed in triplicates to determine the minimal peptide recognized (A). Responses to $S_{813-829}$ and homologous coronavirus peptides prior to vaccination were assessed in 3 donors (B-C). Briefly, cryopreserved PBMCs isolated prior to COVID-19 vaccinations were thawed and IFN-g ELISpot assay was performed with indicated peptides. Lines indicate SFU=20 (B) and SI=3 (C). CD4+ T cell avidity to coronavirus peptides was tested by titrating peptide concentration in the IFN-g ELISpot assay (D-F). Briefly, CD8+ T cell depleted PBMCs were isolated from 3 vaccinated individuals, and an IFN-g ELISpot assay was done in triplicate with $S_{815-827}$ or

homologous peptides titrated serially to determine T cell avidity. Mean of replicates was used to plot spot forming units (SFU) (D-F).

We next asked whether $S_{815\text{-}827}$-specific $CD4^+$ T cells do in fact cross-recognize homologous epitopes from bat coronaviruses. To assess this, we generated T cell lines specific to $S_{815\text{-}827}$ over 10 days. We then restimulated these antigen-specific T cell lines with the same antigen ($S_{815\text{-}827}$) or with homologous peptides from bat coronaviruses, and then we measured cytokine production by intracellular cytokine staining and flow cytometry analysis. As expected, $S_{815\text{-}827}$-specific $CD4^+$ T cells responded robustly to restimulation with the same peptide, with significant increases in the percentage of IFN-γ^+ TNF-α^+ cells as compared with cells that were not cultured with $S_{815\text{-}827}$ for 10 days. Interestingly, restimulation with pep-tides from other coronaviruses also resulted in a robust increase in the percentage of IFN-γ^+ TNF-α^+ cells over control conditions (Figure 4.3), suggesting that some $S_{815\text{-}827}$-specific T cells were cross- reactive. Overall, $S_{815\text{-}827}$-specific $CD4^+$ T cells from all responders produced cytokines when stimulated with bat coronaviruses.

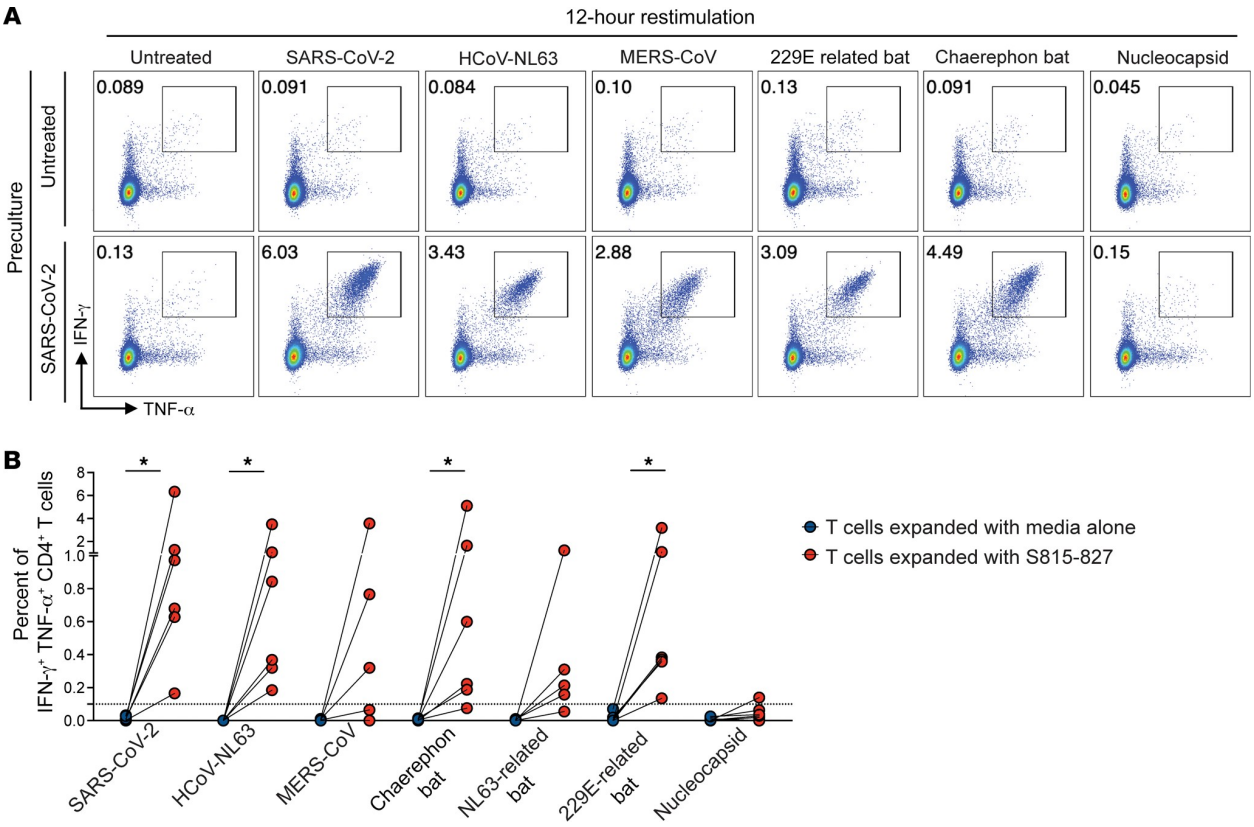

Figure 4.3: $S_{815-827}$ specific CD4+ T cells respond to re-stimulation with homologous peptides from diverse coronaviruses. T cell lines specific for $S_{815-827}$ were generated by expanding PBMCs for 10 days with $S_{815-827}$. Following expansion, cells were re-stimulated for 12-hours with either the same peptide ($S_{815-827}$) or with homologous peptides from diverse coronaviruses and stained for IFN-g and TNF-α expression. Cells were re-stimulated with SARS-CoV-2 nucleocapsid peptide pools as a specificity control. Representative flow plots are shown, with peptides used for expansion indicated on the left, and peptides used for re-stimulation indicated at the top are shown (A). IFN-g+ TNF-α+ CD4+ T cells are gated, with percentages indicated. Responses for all donors tested (n=6) are shown (B). Wilcoxon matched-pairs signed rank test used for statistical comparisons.

To definitively show that vaccinated individuals have true cross-reactive T cells

(meaning the same $CD4^+$ T cell clonotypes recognizing $S_{815-827}$ and homologous bat coronavirus peptides), we performed the ViraFEST assay. The ViraFEST assay uniquely pairs antigen-specific memory T cell responses and their cognate T cell receptors (TCRs), with the specific antigen stimulating this response after a 10-day T cell culture with relevant antigen followed by TCR Vβ CDR3 sequencing (32). We previously used this assay to identify SARS-CoV-2 and HCoV cross-reactive T cells in COVID-19 convalescent patients (19). Cross reactivity is defined by the functional expansion of the same $CD4^+$ TCR clonotypes in response to multiple coronavirus peptides.

We performed the ViraFEST assay using PBMCs from 3 donors (CCP4, VR36, and VR58) and peptides from 6 coronavirus- es (SARS-CoV2, HCoV-NL63, and MERS-CoV and NL63-related bat, 229E-related bat, and Chaerephon bat coronaviruses) (Figure 4.4). In all donors tested, we found multiple cross-reactive T cells that recognized $S_{815-827}$ and homologous bat coronavirus peptides (Figure 4.4 and 4.5). In CCP4, we found TCR clonotypes that recognized the SARS-CoV-2 peptide $S_{815-827}$ and homologous peptides from HCoV-NL63, MERS-CoV, 229E-relat- ed bat virus, and Chaerephon bat coronavirus (Figure 4.4A, indicated in green). Similarly, cross-reactive T cells were observed in VR58 (Figure 4.3B), such as a TCR clonotype that recognized all 6 coronavirus peptides tested (indicated in orange), and in VR36 (Figure 4.4C) such as a TCR clonotype that recognized $S_{815-827}$ and pep tides from HCoV-NL63, 229E-related bat virus, and Chaerephon bat coronavirus (indicated in blue). $CD4^+$ T cell clones specific to NL63-related bat coronavirus peptide were recognized

using the ViraFEST assay for VR36 and VR58, despite these donors having a negative result in IFN-γ ELISpot (Figure 4.1E). This may be because antigen-specific expansion allows for the detection of memory T cell responses that are not picked up by the ELISpot assay.

Since HCoV-HKU1 was recognized by all $S_{815-827}$ responders with the IFN-γ ELISpot assay (Figure 4.1D), we reasoned that cross-reactive clones identified with ViraFEST might also cross-recognize HCoV-HKU1. To test this, we expanded PBMCs from VR36 and 58 using HCoV-HKU1 peptide and performed the ViraFEST assay. We found that some but not all identified cross-reactive clones recognized HCoV-HKU1 (Figure 4.5). Interestingly, we also found cross-reactive TCRs that did not recognize SARS-CoV2 but recognized other coronaviruses (Figure 4.6), suggesting that a subset of cross-reactive T cells might result from priming by prior HCoV exposure.

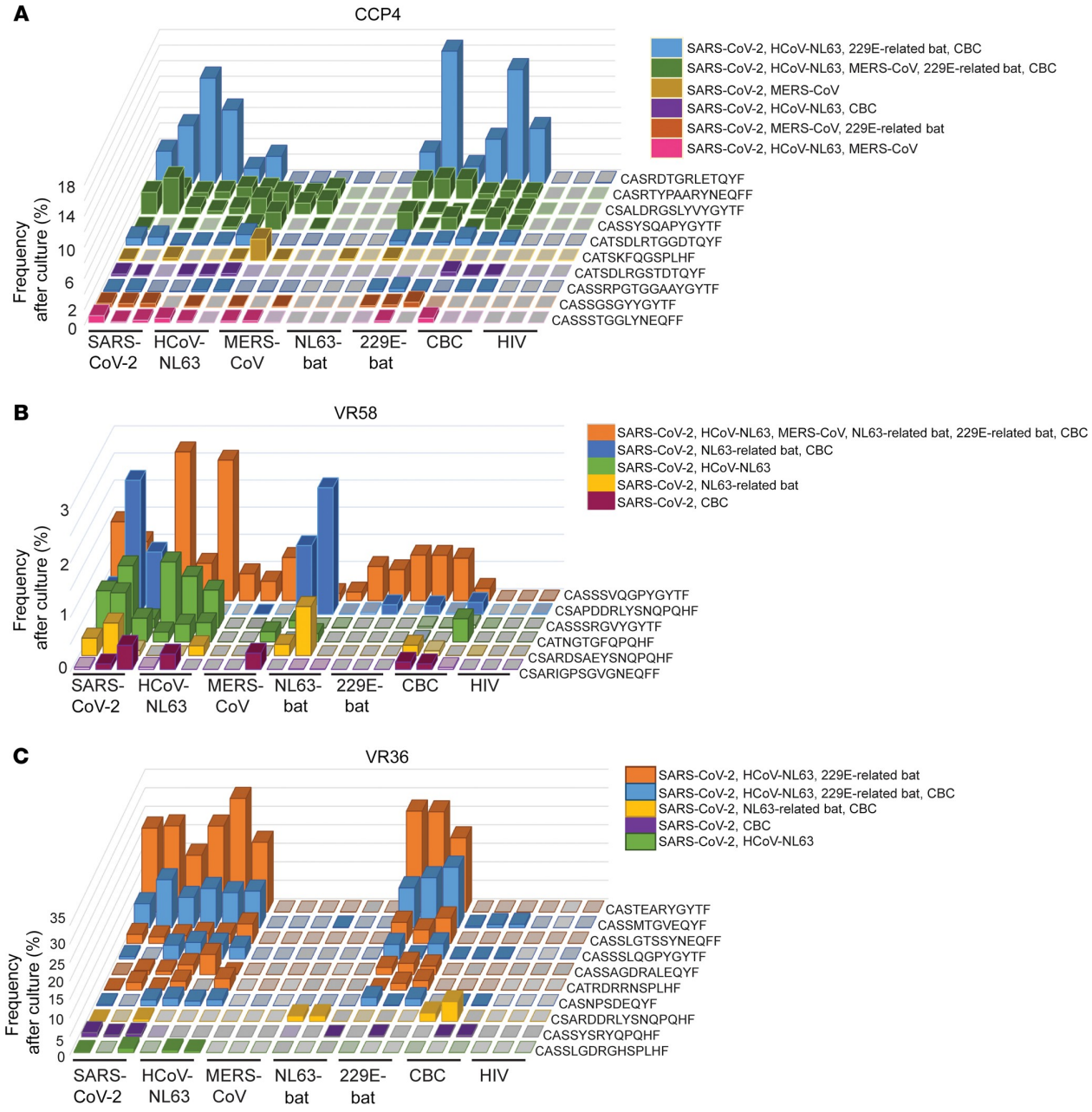

Figure 4.4: CD4+ T cell clonotypes that cross-recognize both $S_{815-827}$ and homologous peptides from diverse coronaviruses are present in vaccinated donors. PBMCs isolated from three donors (CCP4, VR36 and VR58) were expanded for 10 days with $S_{815-827}$ or homologous peptides from HCoV-NL63, MERS-CoV, NL63-related bat, 229E-related bat, and Chaerephon bat coronavirus (CBC). HIV-1 Nef

peptides were included as a specificity control. Following culture, CD4+ T cells were isolated and TCR Vβ CDR3 sequencing was done to identify antigen-specific memory T cells that expanded in response to relevant antigen (Vira-FEST assay). Cross-reactivity was defined by the functional expansion of the same CD4+ TCR clonotypes in response to multiple coronavirus peptides. Peptide co-culture was done in triplicate. Data are shown as the (%) frequency after culture (*y* axis) of antigen-specific $CD4^+$ T cell clonotypes (*z* axis) for all peptide pools tested (*x* axis). Solid colors represent significant clonotypic expansion in response to the indicated antigenic peptide pool(s), whereas translucent colors indicate the clonotype was present at low frequency in the well, but did not significantly expand. Gray colors indicate the relevant TCR clonotype was not detected in that well. Different colors indicate different patterns of cross-reactive T cells shown in a key above each figure. Cross-reactive clones for CCP4 (A), VR58 (B), and VR36 (C) are shown, with different patterns of cross-reactive T cells color coordinated. NL63-Bat = NL63-related Bat, 229E-Bat = 229E-related Bat, CBC = Chaerephon bat coronavirus, HIV = HIV-1 Nef

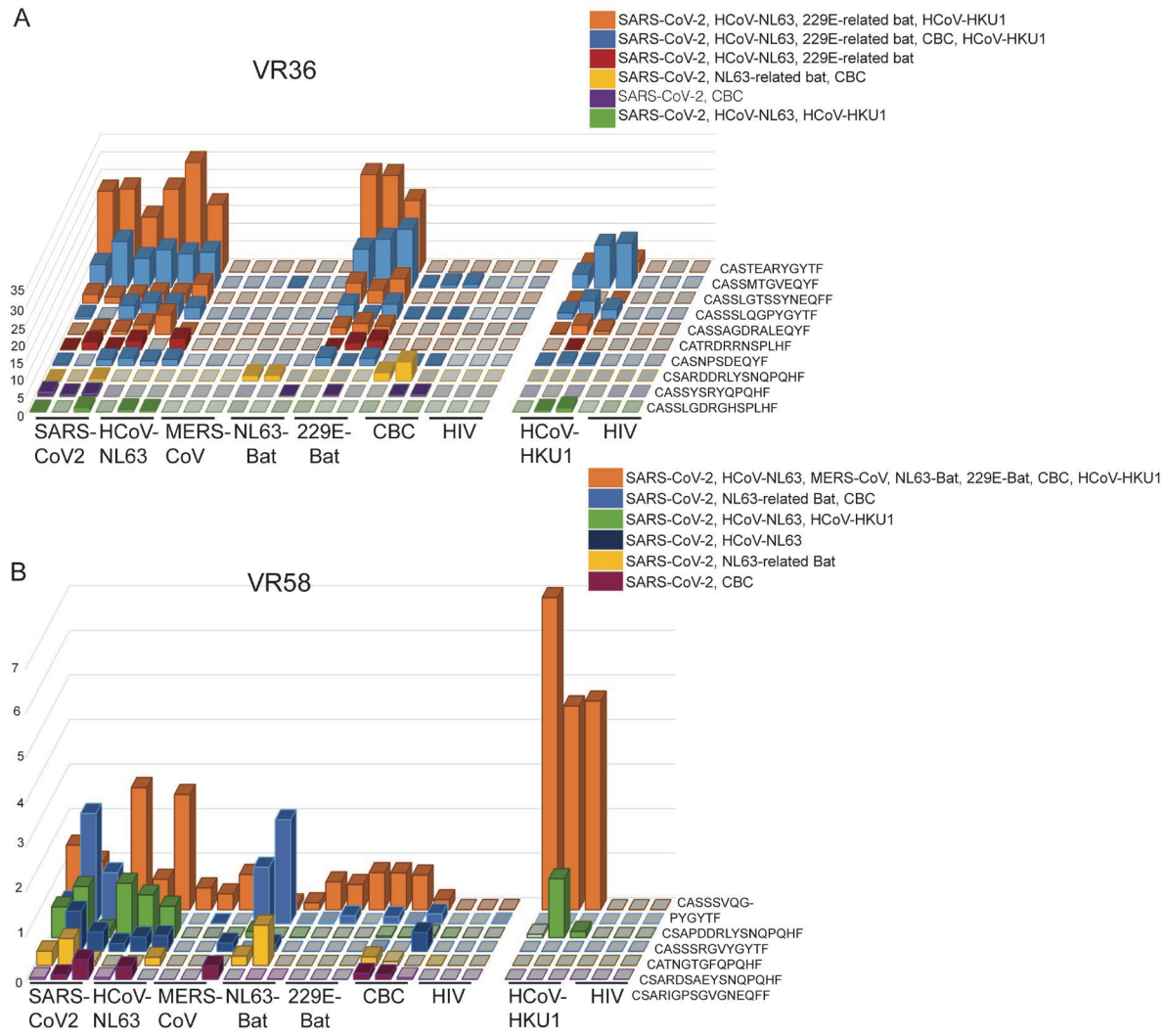

Figure 4.5: Cross-reactive CD4+ T cell clonotypes that react to diverse coronaviruses are found in vaccinated donors. PBMCs were expanded for 10 days with $S_{815\text{-}827}$ or homologous peptides from HCoV-NL63, MERS-CoV, NL63-related bat, 229E-related bat, and Chaerephon bat coronavirus (CBC) (shown on Figure 4.1). On separate timepoint, PBMCs were expanded with HCoV-HKU1. HIV-1 Nef peptides were included as a specificity control at both timepoints. Following culture, CD4+ T cells were isolated and TCR Vβ CDR3 sequencing was done to identify antigen-specific memory T cells that expanded in response to relevant antigen (VIRA-FEST assay). Cross-

reactivity was defined by the functional expansion of the same CD4+ TCR clonotypes in response to multiple coronavirus peptides. Peptide co-culture was done in triplicate. Data are shown as the (%) frequency after culture (*y* axis) of antigen-specific $CD4^+$ T cell clonotypes (*z* axis) for all peptide pools tested (*x* axis). Solid colors represent significant clonotypic expansion in response to the indicated antigenic peptide pool(s), whereas translucent color indicates the clonotype was present at low frequency in the well, but did not significantly expand. Gray indicates the relevant TCR clonotype was not detected in that well. Cross-reactive clones for VR36 (A), and VR58 (B) are shown, with different patterns of cross-reactive T cells color coordinated. NL63-Bat = NL63-related Bat, 229E-Bat = 229E-related Bat, CBC = Chaerephon bat coronavirus, HIV = HIV-1 Nef

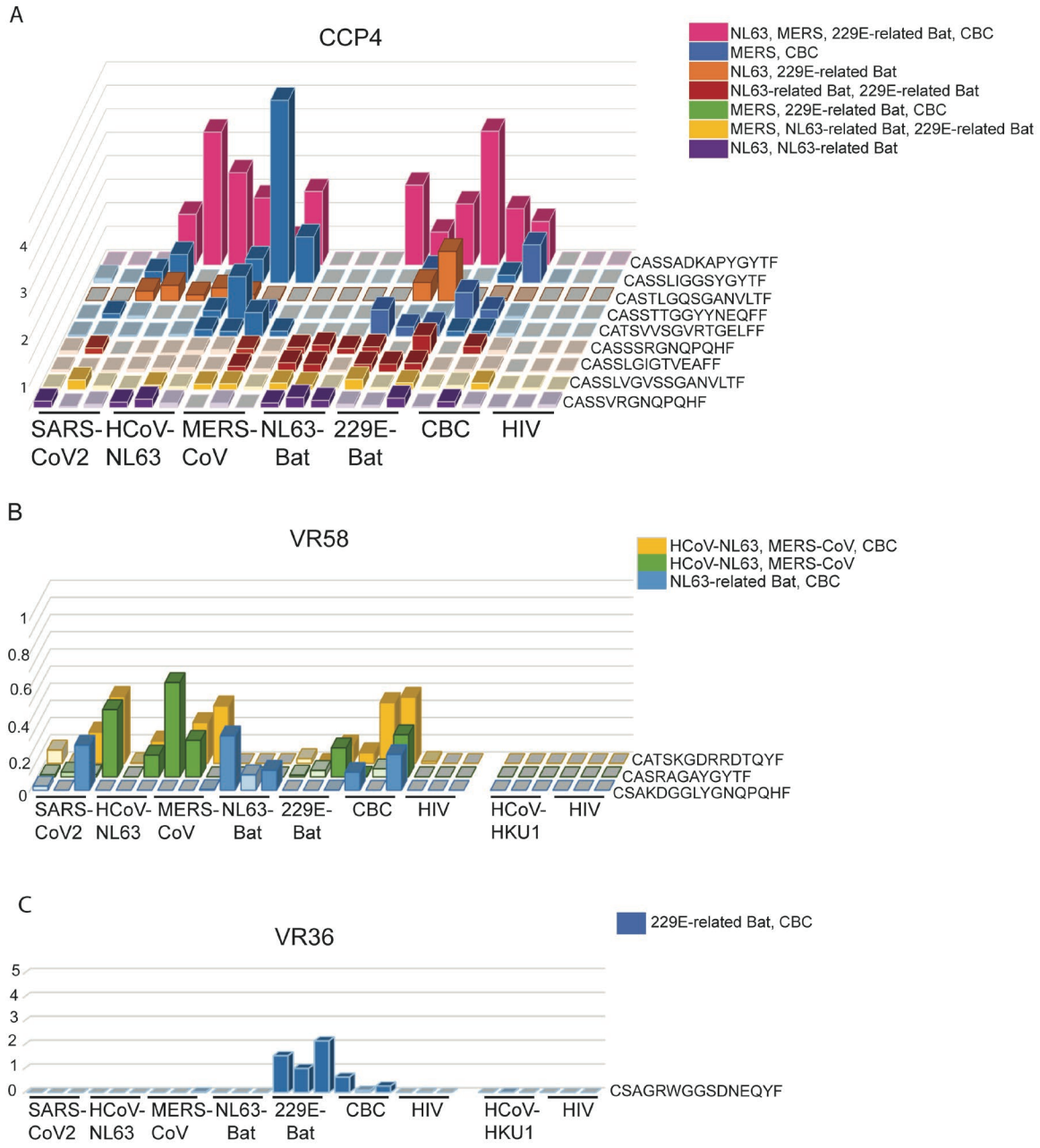

Figure 4.6: Cross-reactive CD4+ T cell clonotypes that react to diverse coronaviruses (excluding $S_{815-827}$) are found in vaccinated donors. PBMCs were expanded for 10 days with $S_{815-827}$ or homologous peptides from HCoV-NL63, MERS-CoV, NL63-related bat, 229E-related bat, and Chaerephon bat coronavirus (CBC). On separate timepoint, PBMCs from VR36 and 58 were expanded with HCoV-HKU1. HIV-1

Nef peptides were included as a specificity control at both timepoints. Following culture, CD4+ T cells were isolated and TCR Vβ CDR3 sequencing was done to identify antigen-specific memory T cells that expanded in response to relevant antigen (VIRA-FEST assay). Cross-reactivity was defined by the functional expansion of the same CD4+ TCR clonotypes in response to multiple coronavirus peptides. Peptide co-culture was done in triplicate. Data are shown as the frequency (%) after culture (*y* axis) of antigen-specific $CD4^+$ T cell clonotypes (*z* axis) for all peptide pools tested (*x* axis). Solid bars represent significant clonotypic expansion in response to the indicated antigenic peptide pool(s), whereas translucent color indicates the clonotype was present at low frequency in the well, but did not significantly expand. Gray indicates the relevant TCR clonotype was not detected in that well. Colors indicate different patterns of cross-reactive T cells. Cross-reactive clones for CCP4 (A), VR58 (B), and VR36 (C) are shown, with different patterns of cross-reactive T cells color coordinated. NL63-Bat = NL63-related Bat, 229E-Bat = 229E-related Bat, CBC = Chaerephon bat coronavirus, HIV = HIV-1 Nef

Table 4.1: List of coronaviruses peptides and sequences used in this study

#	Name	Host	Genus	Subgenus	Accession #	Sequence
1	SARS CoV 2	Human	Betacoronavirus	Sarbecovirus	MN908947.3	RSFIEDLLFNKVT
2	SARS CoV	Human	Betacoronavirus	Sarbecovirus	P59594	RSFIEDLLFNKVT
3	MERS CoV	Human	Betacoronavirus	Merbecovirus	AKN11071	RSAIEDLLFDKVT
4	HCoV NL63	Human	Alphacoronavirus	Setracovirus	APF29063	RSALEDLLFSKVV
5	HCoV 229E	Human	Alphacoronavirus	Duvinacovirus	AGT21367	RSAIEDILFSKLV
6	HCoV OC43	Human	Betacoronavirus	Embecovirus	AXX83381	RSAIEDLLFDKVK
7	HCoV HKU1	Human	Betacoronavirus	Embecovirus	AYN64561	RSLLEDLLFNKVK
8	Chaerephon bat	Bat	Alphacoronavirus	unclassified	ADX59495.1	RSFIEDLLFDKVV
9	NL63-related bat	Bat	Alphacoronavirus	Setracovirus	APD51483.1	RSFVEDLLFDKVV
10	229E-related bat	Bat	Alphacoronavirus	Duvinacovirus	ALK28767.1	RSAIEDILFSKVV
11	Feline Coronavirus	Feline	Alphacoronavirus	Tegacovirus	ADC35472.1	RSAVEDLLFNKVV

Table 4.2: S815-827 is identical in sequence between SARS-CoV-2 and SARS-related coronaviruses in the subgenus sarbecovirus and genus betacoronavirus listed.

Virus Name	Host species	Genus	Subgenus	Sequence	Residue	Accession	Source DOI
SARSCoV 2	Human	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	815-827	MN908947.3	10.1038/s41586-020-2008-3
Longquan_140	Rhinolophus_monoceros	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	784-796	KF294457	10.1016/j.virol.2017.03.019
RaTG13	Rhinolophus_affinis	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	811-823	MN996532	10.1038/s41586-020-2012-7
CoVZC45	Rhinolophus_sinicus	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	788-800	MG772933	10.1038/s41426-018-0155-5
CoVZXC21	Rhinolophus_sinicus	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	787-799	MG772934	10.1038/s41426-018-0155-5
GX-P4L	Manis_javanica	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	809-821	MT040333	10.1038/s41586-020-2169-0
RacCS203	Rhinolophus_acuminatus	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	787-799	MW251308	10.1038/s41467-021-21240-1
Rc-o319	Rhinolophus_cornutus	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	777-789	LC556375	10.3201/eid2612.203386
RpYN06	Rhinolophus_pusillus	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	788-800	MZ081381	10.1016/j.cell.2021.06.008
PrC31	Rhinolophus_spp	Betacoronavirus	Sarbecovirus	RSFIEDLLFNKVT	788-800	MW703458	10.1080/22221751.2021.1964925

Table 4.3: Class II HLA alleles of vaccinated individuals who responded to $S_{815-827}$.

Donor	Vaccine Type	Days since 2nd dose	HLA Allele	Predicted MHC-II Allele (IEDB analysis)	# of positive coronavirus peptides
VR17	BNT162b2	195	DRB1*04:01, 07:01 DRB4*01:03, 01:03N DQA1*03:01, 02:01 DQB1*03:02, 03:03 DPA1*01:03 DPB1*03:01, 04:02	DPA1*01:03/DPB1*04:02	7
VR 25	BNT162b2	150	DRB1*07:01, 13:01 DRB3* 02:02 DRB4*01:01 DQA1*02:01, 01:03 DQB1*02:02, 06:03 DPA1*01:03, 02:02 DPB1*01:01, 02:01	DPA1*02:02/DPB1*01:01 DPA1*02:02/DPB1*02:01 DPA1*01:03/DPB1*01:01 DPA1*01:03/DPB1*02:01	2
VR36	BNT162b2	142	DRB1*01:01, 15:01 DRB5*01:01 DQA1*01:01, 01:03 DQB1*05:01, 06:01 DPA1*01:03 DPB1*04:01	DPA1*01:03/DPB1*04:01	8
VR40	mRNA-1273	272	DRB1*03:01, 15:01 DRB3*01:01 DRB5*01:01 DQA1*05:01, 01:02 DQB1*02:01, 06:02 DPA1*01:03 DPB1*04:01, 06:01	DPA1*01:03/DPB1*04:01 DPA1*01:03/DPB1*06:01	9

VR 41	BNT162b2	159	DRB1*07:01,11:01 DRB3*02:02 DRB4*01:03N DQA1*02:01, 05:05 DQB1*03:03, 03:01 DPA1*01:03, 02:01 DPB1*13:01, 04:01	DPA1*01:03/DPB1*04:01 DPA1*02:01/DPB1*04:01 DPA1*02:01/DPB1*13:01 DPA1*01:03/DPB1*13:01	4
VR58	BNT162b2	89	DRB1*01:03, 13:05 DRB3* 02:02 DQA1*01:01, 05:05 DQB1*05:01, 03:01 DPA1*01:03 DPB1*02:01, 04:01	DPA1*01:03/DPB1*04:01 DPA1*01:03/DPB1*02:01	7
VR60	BNT162b2	73	DRB1*01:03, 13:02 DRB3*01:01, 03:01 DQA1*05:01, 01:02 DQB1*02:01, 06:04 DPA1*01:03 DPB1*02:01	DPA1*01:03/DPB1*02:01	2
VR80	BNT162b2	14	DRB1*07:01, 12:01 DRB3* 02:02 DRB4* 01:01 DQA1*02:01, 05:05 DQB1*02:02, 03:01 DPA1*01:03, 01:04 DPB1*02:01, 15:01	DPA1*01:04/DPB1*02:01 DPA1*01:03/DPB1*15:01 DPA1*01:04/DPB1*15:01 DPA1*01:03/DPB1*02:01	9
VR82	BNT162b2	187	DRB1*04:04, 13:01 DRB3*01:01 DRB4*01:03 DQA1*03:01, 01:03 DQB1*03:02, 06:03 DPA1*01:03 DPB1*06:01, 02:01	DPA1*01:03/DPB1*02:01 DPA1*01:03/DPB1*06:01	7

VR84	BNT162b2	100	DRB1*04:01, 13:01 DRB3 *02:02 DRB4* 01:03 DQA1*03:01, 01:03 DQB1*03:02, 06:03 DPA1*01:03 DPB1*02:01, 04:02	DPA1*01:03/DPB1*04:02 DPA1*01:03/DPB1*02:01	4
VR89	mRNA-1273	98	DRB1*01:02, 07:01 DRB4*01:03 DQA1*01:01, 02:01 DQB1*05:01, 02:02 DPA1*01:03, 02:01 DPB1*02:01, 11:01	DPA1*02:01/DPB1*02:01 DPA1*02:01/DPB1*11:01 DPA1*01:03/DPB1*11:01 DPA1*01:03/DPB1*02:01	6
VR90	BNT162b2	105	DRB1*04:04, 07:01 DRB4*01:03, 01:03N DQA1*03:01, 02:01 DQB1*03:02, 03:03 DPA1*01:03 DPB1*02:01, 04:01	DPA1*01:03/DPB1*04:01 DPA1*01:03/DPB1*02:01	5
CCP3	mRNA-1273	78	DRB1*11:04, 13:03 DRB3*01:62, 02:02 DQA1*05:05, 03:03 DQB1*03:01, 04:02 DPA1*01:03, 02:02 DPB1*04:01, 04:02	DPA1*02:02/DPB1*04:02 DPA1*01:03/DPB1*04:01 DPA1*02:02/DPB1*04:01 DPA1*01:03/DPB1*04:02	6
CCP4	mRNA-1273	77	DRB1*03:01, 04:02 DRB3*01:01 DRB4* 01:03 DQA1*05:01, 03:01 DQB1*02:01, 03:02 DPA1*01:03 DPB1*04:01	DPA1*01:03/DPB1*04:01	10

CCP6	BNT162b2	161	DRB1*03:01 DRB3*01:01 DQA1*05:01 DQB1*02:01 DPA1*01:03 DPB1*04:01	DPA1*01:03/DPB1*04:01	8

4.5 Discussion

Cross-reactive CD4+ T cells that can cross-recognize SARS-CoV-2 and endemic common cold coronaviruses (HCoVs) have been demonstrated in COVID-19 unexposed donors, COVID-19 recovered individuals, and vaccine recipients (19-28). Recent evidence suggests that pre-existing HCoV / SARS-CoV-2 cross-reactive T cells in unexposed individuals might lead to better outcomes after COVID-19 infections (33), possibly because cross-reactive memory T cells have faster re-activation and kinetics that allow for robust responses to acute SARS-CoV-2 infection (31).

In this study, we looked at T cell responses to a highly conserved region of SARS-CoV-2 spike ($S_{815-827}$) in COVID-19 mRNA vaccine recipients. $S_{815-827}$ is a highly conserved epitope in alpha and betacoronaviruses (29), and is identical in sequence in coronaviruses closely related to SARS-CoV-2 (Table 4.2). This degree of conservation suggests that $S_{815-827}$ has an important functional role and might be less likely to be impacted by escape mutations, making it an appealing target for vaccine strategies.

Our results, using IFN-γ ELISpot, show that 40% of vaccinated participants in our cohort mount T cell responses to $S_{815-827}$, suggesting that a significant percentage of the population might have T cells reactive to this conserved coronavirus epitope. This is

consistent with a recent report from Loyal *et al* that showed that the 15-mer peptide $S_{816-830}$ is immunodominant and is recognized by most vaccinated individuals (31). In their cohort, Loyal et al showed that $S_{816-830}$ is targeted by 90% of vaccinated individuals using the activation induced marker (AIM+) assay, which detects antigen specific T cell activation regardless of cytokine production and expansive capacity. The AIM+ assay is likely more sensitive than IFN-γ ELISpot, leading to a higher percentage of vaccinated individuals recognizing the conserved epitope in Loyal et al. Furthermore, in our study, we might have underestimated the percentage of $S_{816-830}$ responders because we looked at T cell responses in vaccinees up to 272 days post vaccination, and T cell responses may have waned.

In their cohort, Loyal et al have showed that $S_{816-830}$ is recognized by only 20% of unexposed donors, versus 50% of COVID19 convalescent patients and 90% of vaccinated individuals (31), suggesting that in most cases, $S_{815-827}$ reactive T cells are induced or expanded by COVID-19 exposure. This is consistent with the fact that we did not find T cell responses to $S_{815-827}$ with IFN-γ ELISpot in matched pre-vaccine samples from 3 study participants who had CD4+ T cells post-vaccination. However, it is worth noting that our observation might be limited by reduced sensitivity of the IFN-γ ELISpot assay in pre-vaccine samples for which lower cell numbers were used due to limited cell availability. Furthermore, using the ViraFEST assay, we identified CD4+ T cell clonotypes that are cross-reactive to HCoVs and bat coronaviruses but do not recognize SARS-CoV-2 (Figure 4.6), suggesting that a subset of cross-reactive T cells might result from priming by prior HCoV exposure.

To our knowledge, no prior study has identified cross-reactive T cells that recognize peptides from bat coronaviruses. In our study, we show that most $S_{815-827}$ responders also recognized peptides from at least six other $S_{815-827}$ homologous coronavirus peptides ex-vivo. Furthermore, we show that $S_{815-827}$ specific T cell lines produce cytokines in response to re-stimulation with homologous peptides from bat coronaviruses. Finally, we identify truly cross-reactive T cells, by identifying CD4+ TCR clonotypes that functionally expand in response to both $S_{815-827}$ and homologous bat coronaviruses with the ViraFEST assay. This provides evidence that some vaccinated individuals harbor SARS-CoV-2 and bat coronavirus cross-reactive T cells.

Given the threat posed by future coronavirus pandemics, the development of pan-coronavirus strategies that can enhance protection against potentially zoonotic coronaviruses has garnered increased interest (7). Wang et al has shown an S2 fusion domain antibody that can cross-neutralize betacoronaviruses including MERS-CoV in animal models (34). Neutralizing antibodies targeting S2 fusion domain have also been described in CCPs (35) and have been shown to cross-neutralize other betacoronaviruses (36). Additionally, it's been shown that BNT162b2 immunized individuals with prior SARS-CoV exposure develop antibodies that can cross-neutralize other sarbecoviruses (37). Collectively, these studies suggest that it might be possible to induce immunity against potentially zoonotic coronaviruses. However, to our knowledge, T cell responses to potentially zoonotic coronaviruses have not yet been studied.

Our results suggest that a large percentage of individuals who received COVID-19 mRNA vaccines have T cells that recognize bat coronavirus peptides, likely due to

cross-reactive T cells that target $S_{815-827}$ and homologous bat coronavirus peptides. Additionally, we show that genetically diverse bat coronaviruses from the beta and alphacoronavirus genus can also be cross-recognized by T cells from vaccinated individuals. Our data support the hypothesis that current COVID-19 vaccinations might enhance protection against certain SARS-CoV-2 related bat coronaviruses. Further, our results provide insight into the development of pan-coronavirus vaccine strategies, such as mRNA vaccines that code for multiple diverse coronavirus peptides, that might have the ability to induce protection against multiple coronaviruses.

4.6 References

1. WHO COVID-19 Dashboard. Geneva: World Health Organization, 2020. https://covid19.who.int/. Accessed Oct 12, 2021.
2. Zhu N, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med.* 2020;382(8):727-733.
3. Gorbalenya AE, et al. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology.* 2020;5(4):536-544.
4. Wu A, et al. Genome Composition and Divergence of the Novel Coronavirus (2019-nCoV) Originating in China. *Cell Host Microbe.* 2020;27(3):325-328.
5. Zhou P, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* 2020;579(7798):270-273.
6. Lytras S, et al. The animal origin of SARS-CoV-2. *Science.* 2021;373(6558):968-970.

7. Morens DM, et al. Universal Coronavirus Vaccines — An Urgent Need [published online December 15, 2021]. *N Engl J Med.* https://doi.org/10.1056/NEJMp2118468.
8. Polack FP, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med.* 2020;383(27):2603-2615.
9. Baden LR, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med.* 2021;384(5):403-416.
10. Voysey M, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet.* 2021;397(10269):99-111.
11. Sadoff J, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *N Engl J Med.* 2021;384(23):2187-2201.
12. Sahin U, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. *Nature.* 2020;586(7830):594-599.
13. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in infected or vaccinated individuals. *Cell Rep Med.* 2021;2(7):100355.
14. Woldemeskel BA, et al. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. *J Clin Invest.* 2021;131(10):e149335.
15. Wyllie D, et al. SARS-CoV-2 responsive T cell numbers are associated with protection from COVID-19: A prospective cohort study in keyworkers [preprint].

https://doi.org/10.1101/2020.11.02.20222778. Posted on *medRxiv November 04,* 2020.

16. Zhao J, et al. Airway Memory CD4(+) T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity.* 2016;44(6):1379-1391.
17. Zhao J, et al. T cell responses are required for protection from clinical disease and for virus clearance in severe acute respiratory syndrome coronavirus-infected mice. *J Virol.* 2010;84(18):9318-9325.
18. McMahan K, et al. Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature.* 2021;590(7847):630-634.
19. Dykema AG, et al. Functional characterization of CD4+ T cell receptors crossreactive for SARS-CoV-2 and endemic coronaviruses. *J Clin Invest.* 2021;131(10):e146922.
20. Grifoni A, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell.* 2020;181(7):1489-1501.e15.
21. Le Bert N, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature.* 2020;584(7821):457-462.
22. Mateus J, et al. Selective and cross-reactive SARS-CoV-2 T cell epitopes in unexposed humans. *Science.* 2020;370(6512):89-94.
23. Braun J, et al. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. *Nature.* 2020;587(7833):270-274.
24. Woldemeskel BA, et al. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. *J Clin Invest.* 2020;130(12):6631-6638.

25. Nelde A, et al. SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nat Immunol.* 2021;22(1):74-85.
26. Bacher P, et al. Low-Avidity CD4(+) T Cell Responses to SARS-CoV-2 in Unexposed Individuals and Humans with Severe COVID-19. *Immunity.* 2020;53(6):1258-1271.e5.
27. Richards KA, et al. Circulating CD4 T Cells Elicited by Endemic Coronaviruses Display Vast Disparities in Abundance and Functional Potential Linked to Antigen Specificity and Age. *J Infect Dis.* 2021;223(9):1555-1563.
28. Sekine T, et al. Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. *Cell.* 2020;183(1):158-168.e14.
29. Robson B. COVID-19 Coronavirus spike protein analysis for synthetic vaccines, a peptidomimetic antagonist, and therapeutic drugs, and analysis of a proposed achilles' heel conserved region to minimize probability of escape mutations and drug resistance. *Comput Biol Med.* 2020;121:103749.
30. Ong E, Huang X, Pearce R, Zhang Y, He Y. Computational design of SARS-CoV-2 spike glycoproteins to increase immunogenicity by T cell epitope engineering. *Computational and Structural Biotechnology Journal.* 2021;19:518-529.
31. Loyal L, et al. Cross-reactive $CD4^+$ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination. *Science.* 2021;374(6564):eabh1823.
32. Danilova L, et al. The Mutation-Associated Neoantigen Functional Expansion of Specific T Cells (MANAFEST) Assay: A Sensitive Platform for Monitoring Antitumor Immunity. *Cancer Immunol Res.* 2018;6(8):888-899.

33. Mizgerd, et al. Recent endemic coronavirus infection is associated with less-severe COVID-19. *J Clin Invest.* 2021;131(1).
34. Wang C, et al. A conserved immunogenic and vulnerable site on the coronavirus spike protein delineated by cross-reactive monoclonal antibodies. *Nature Communications.* 2021;12(1):1715.
35. Poh CM, et al. Two linear epitopes on the SARS-CoV-2 spike protein that elicit neutralising antibodies in COVID-19 patients. *Nature communications.* 2020;11(1):2806.
36. Hurlburt NK, et al. Structural definition of a pan-sarbecovirus neutralizing epitope on the spike S2 subunit [preprint]. https://doi.org/10.1101/2021.08.02.454829. Posted on *bioRxiv. August 03,* 2021.
37. Tan C, et al. Pan-Sarbecovirus Neutralizing Antibodies in BNT162b2-Immunized SARS-CoV-1 Survivors. *N Engl J Med.* 2021;385(15):1401-1406.
38. Chan HY, et al. A T cell receptor sequencing-based assay identifies cross-reactive recall CD8+.T cell clonotypes against autologous HIV-1 epitope variants/ Front Immunol. 2020;11:1-9.
39. Wang P, et al. Peptide binding predictions for HLA DR, DP and DQ molecules. *BMC Bioinformatics.* 2010;11(1):568.
40. Wang P, et al. A Systematic Assessment of MHC Class II Peptide Binding Predictions and Evaluation of a Consensus Approach. *PLOS Computational Biology.* 2008;4(4):e1000048.

Contributions and Acknowledgments:

This chapter was published in the Journal of Clinical Investigation (Woldemeskel BA, Dykema AG, Garliss C, Cherfils S, Smith KN, Blankson JN. CD4+ T-cells from COVID-19 mRNA vaccine recipients recognize a conserved epitope present in diverse coronaviruses. *J Clin Invest.* 2022. doi:10.1172/JCI156083).

Data acquisition of flow cytometry experiments were done by Caroline Garliss. ViraFEST experiments were done in collaboration with Dr. Kellie Smith's group, with Arbor Dykema acquiring and analyzing sequencing data. Saphira Cherfills assisted in the analysis of ELISpot data using excel.

5 Chapter 5: mRNA vaccine-elicited SARS-CoV-2-specific T cells persist at 6 months and recognize the delta variant

5.1 Abstract

Little is known about the decay kinetics of coronavirus disease 2019 vaccine–elicited severe acute respiratory syndrome coronavirus 2–specific T cells. In this study we show a modest decline in the frequency of these T cells at 6 months and demonstrate robust expansion in response to antigen and recognition of spike peptides from the Delta variant.

5.2 Introduction

Recent studies have shown a decline in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–specific neutralizing antibody titer 6 months after receipt of mRNA vaccines [1]. In contrast, little is known about the rate of decay of vaccine-elicited T cells. T cells protect against reinfection in macaques [2] and have been shown to recognize the Alpha and Beta variants as effectively as the vaccine strain [3–5], thereby

possibly providing protection against severe disease. Given the important role of these cells, it will be important to determine how long they persist after vaccination. In this study, we compared the frequency of SARS CoV- 2–specific T-cell responses at 2 weeks and at 6 months postvaccination and measured the response of T cells to Delta variant spike peptides at 6 months. Our results have implications for vaccine boosting strategies.

5.3 Methods

We obtained blood from 21 study participants (13 men, 8 women). For 15 of these participants (10 men, 5 women), we obtained blood prior to vaccination and postvaccination at 7–14 days and 6 months after the second dose of the vaccine. Fourteen of these participants received the Pfizer-BioNTech (BNT162b2) vaccine and 1 received the Moderna (mRNA-1273) vaccine.

The median age of the participants was 41 years (range: 23 to 56 years). Informed consent was obtained from all study participants. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll centrifugation. Plasma was used to screen for natural infection with SARS-CoV-2 by testing for antibodies to the nucleocapsid protein with the Bio-Rad Platelia SARS-CoV-2 Total Antibody assay (Marnes-la-Coquette, France). We determined cellular immunity to the SARS-CoV-2 spike protein by performing an interferon-γ (IFN-γ) Elispot assay with unfractionated PBMCs that were stimulated with a pool of overlapping SARS-CoV-2 spike peptides (BEI, Manassas, VA), as previously described [3]. The assay was also performed with CD8+ T-cell–depleted PBMCs to determine the relative contribution of CD4+ T cells and

CD8+ T cells to the cellular immune response. To compare recognition of spike proteins from the vaccine strain and the Delta variant, we stimulated PBMCs with overlapping spike peptide pools from both viruses at a concentration of 1 μg/mL (JPT, Berlin, Germany). To determine how effectively SARS-CoV-2– specific T cells proliferate in response to the vaccine strain spike peptide, PBMCs were stimulated with overlapping peptides or media alone in standard media with 10 units/mL of interleukin 2 (IL-2) for 10–12 days and then washed and rested overnight in media alone for 24 hours. The cells were then stimulated for 12 hours in the presence of protein transport inhibitors (GolgiPlug, 1 μg/mL; GolgiStop, 0.7 μg/mL) and antibodies against CD28 and CD49d (all from BD Biosciences, Franklin Lakes, NJ) and then stained with annexin V (BD Biosciences) and antibodies to CD3 and CD4 (Biolegend) and then fixed and permeabilized and stained with antibodies to IFN-γ and tumor necrosis factor α (TNF-α; both from BD Biosciences). Flow cytometry was performed on a BD FACS LSRFortessa flow cytometer, and data were analyzed using FlowJo software, version 10. Data on a minimum of 100 000 events in the lymphocyte gate were collected and analyzed. All statistical analyses were performed using GraphPad Prism Software (version 9.2.0). For experiments requiring comparisons between 2 groups, a 2-tailed paired Student's t test was used to determine significance. For experiments requiring multiple comparisons, a Friedman test with Dunn's multiple comparison test was used. A P value of less than .05 was considered statistically significant.

5.4 Results

We previously determined that the frequency of T cells that recognized spike peptides was extremely low prior to vaccination, with a median of 2.7 spot forming units (SFU)

per million PBMCs [3]. This frequency had increased to a median of 237 SFU per million PBMCs at day 7 to day 14 postvaccination [3]. At 6 months, the SFU had decreased to 122 SFU per million PBMCs, a number that was still significantly higher than the prevaccination level (day 0) (Figure 5.1A). In order to determine whether these responding cells were predominantly CD4+ or CD8+ T cells, we depleted CD8+ T cells from PBMCs and repeated the Elispot assay. As reported earlier, depletion of CD8+ T cells resulted in higher responses, suggesting that the responses were predominantly driven by CD4+ T cells. There was a median of 260 SFU per million cells at 7 to 14 days postvaccination [3], and at 6 months the response had declined to 166 SFU per million cells (Figure 5.1B). This number was significantly higher than the prevaccination level and not statistically different from the frequency present at days 7 to 14. We asked whether the

cells present at the 6-month time point were capable of proliferating in response to stimulation with the spike peptides. To do this, we cultured PBMCs with spike peptides or media alone for a 10- to 12-day period to allow for the cells to proliferate, followed by restimulation with the peptide for 12 hours in order to induce cytokine expression. mRNA for the nucleocapsid gene is not included in the vaccines; therefore, we used a pool of peptides from this protein as a specificity control. As shown in Figure 5.1C, there was a low frequency of CD4+ T cells that co-expressed TNF-α and IFN-γ in response to spike peptides after the cells had been cultured with media alone (median of 0.01%). In contrast, after stimulation with spike peptides for 10 days, there was a marked increase in the frequency of spike specific CD4+ T cells (median of 2.37%). There was no significant difference in the frequency of expanded cells that were detected at days 7–

14 and 6 months postvaccination (Figure 5.1D). Finally, we asked whether the spike-specific cells present at 6 months postvaccination would also recognize spike proteins from the Delta variant virus, which is currently the predominant circulating virus. As shown in Figure 5.1E, there was no significant difference in the frequency of T cells that recognized spike peptide pools from the vaccine strain versus the Delta variant (median of 54.6 SFU vs 82 SFU per million PBMCs,

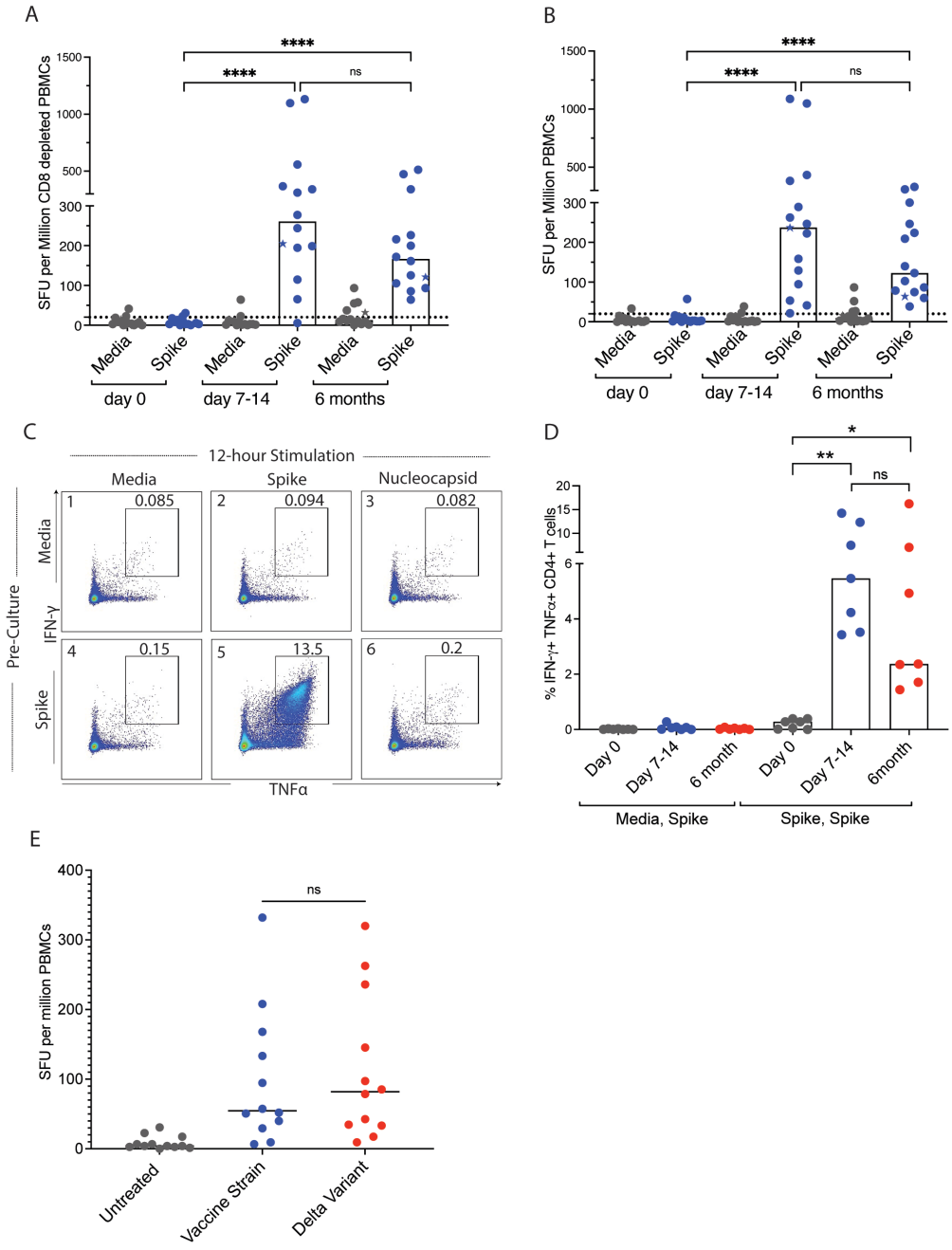

Figure 5.1: T cell responses of COVID-19 mRNA vaccinated individuals at different timepoints. The number of SFU per million cells generated in response to stimulation with spike peptides is shown at different time points for unfractionated PBMCs (A) and CD8-depleted PBMCs (B). Values from study participants who received

the BNT162b2 vaccine are shown as circles and values from the study participant who received the mRNA-1273 are shown as a star. Horizontal bars represent the median value. (C) Flow cytometry plot showing co-expression of TNF-α and IFN-γ of CD4+ T cells after 10–12-day preculture with either media alone or with SARS-CoV-2 spike peptides followed by 12-hour stimulation with media alone, spike peptides, or nucleocapsid peptides. (D) The percentage of cells co-expressing both cytokines in response to media preculture followed by spike peptide stimulation (media, spike) versus spike peptide preculture and spike peptide stimulation (spike, spike) at different time points. (E) The number of SFU generated in response to stimulation with spike peptides from the vaccine strain or Delta variant viruses. 0.1234 (ns), 0.0332 (∗), 0.0021 (∗∗), 0.0002 (∗∗∗), <0.0001 (∗∗∗∗). Abbreviations: IFN-γ, interferon-γ; ns, nonsignificant; PBMC, peripheral blood mononuclear cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot forming units; TNFα, tumor necrosis factor α.

5.5 Discussion

Recent studies have shown a decline in the titer of neutralizing antibodies 6 months after vaccination [1]. Furthermore, the Delta variant, which is the predominant variant of concern currently in circulation, is not fully neutralized by antibodies generated by current mRNA vaccines [6, 7]. These 2 factors may partially explain breakthrough infections of vaccinated individuals, especially given the correlation of breakthrough infections with lower neutralizing antibody titers [8].

mRNA vaccines elicit strong SARS-CoV-2–specific T-cell responses [9, 10]. Studies have suggested that SARS-CoV-2– specific T cells play a protective role in natural

infection and in vaccinated individuals [11]. Direct evidence for a role of T cells in protection comes from studies showing that depletion of CD8+ T cells in convalescent rhesus macaques partially abrogates the resistance of these animals to SARS-CoV-2 reinfection [2]. However, it is not known how long vaccine-induced T cells remain in circulation. Naive T cells normally expand in response to recognition of cognate antigen and develop into effector and memory T cells. The frequency of effector T cells will decline with time, but a low percentage of memory cells will persist and expand if the same antigen is encountered again.

Here we showed a modest decline in total and SARS-CoV-2– specific T cells 6 months after vaccination. These cells expanded to much higher frequencies following a 10-day culture period, which is consistent with a memory T-cell phenotype. We also show that vaccine-generated T cells recognize spike peptides from the Delta variant as efficiently as spike peptides from the vaccine strain, which is consistent with prior studies showing that T cells recognize many epitopes in the spike protein that are not affected by mutations present in the Alpha and Beta variants [3–5]. The persistence of T cells that can recognize variants of concern could potentially explain the protection from severe disease that has been reported in vaccine breakthrough infections [12].

Our study is limited by a relatively small number of study participants, but by analyzing longitudinal samples we show preservation of T-cell responses in vaccinated individuals at 6 months and we also demonstrate efficient T-cell recognition

of the Delta variant spike peptides. Our data further the understanding of the immune response to the SARS-CoV-2 mRNA vaccines. The robust expansion of T cells in

response to stimulation with spike peptides suggests that booster shots should successfully increase the frequency of SARS-CoV-2–specific T cells in circulation.

5.6 References

1. Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. N Engl J Med 2021. doi: 10.1056/
2. NEJMoa2114583.
3. McMahan K, Yu J, Mercado NB, et al. Correlates of protection against SARSCoV-2 in rhesus macaques. Nature 2021; 590:630–4.
4. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and
5. HCoV-NL63. J Clin Invest 2021; 131:e149335.
6. Redd AD, Nardin A, Kared H, et al. CD8+ T-cell responses in COVID-19 convalescent individuals target conserved epitopes from multiple prominent SARSCoV-2 circulating variants. Open Forum Infect Dis 2021; 8:ofab143.
7. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals. Cell Rep
8. Med 2021; 2:100355.
9. Planas D, Veyer D, Baidaliuk A, et al. Reduced sensitivity of SARS-CoV-2 variant Delta to antibody neutralization. Nature 2021; 596:276–80.
10. Liu C, Ginn HM, Dejnirattisai W, et al. Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. Cell 2021; 184:4220–36, e13.
11. Bergwerk M, Gonen T, Lustig Y, et al. Covid-19 breakthrough infections in vaccinated health care workers. N Engl J Med 2021. doi: 10.1056/NEJMoa2109072.

12. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020; 586:594–9.
13. Jackson LA, Anderson EJ, Rouphael NG, et al; mRNA-1273 Study Group. An mRNA vaccine against SARS-CoV-2—preliminary report. N Engl J Med 2020;
14. 383:1920–31.
15. Bertoletti A, Le Bert N, Qui M, Tan AT. SARS-CoV-2-specific T cells in infection and vaccination. Cell Mol Immunol 2021; 18:2307–12.
16. Thompson MG, Burgess JL, Naleway AL, et al. Prevention and attenuation of Covid-19 with the BNT162b2 and mRNA-1273 vaccines. N Engl J Med 2021; 385:320–9.

Contributions and Acknowledgments:

This chapter was published in Clinical Infectious Diseases (Woldemeskel BA, Garliss CC, Blankson JN. mRNA Vaccine-Elicited SARS-CoV-2-Specific T cells Persist at 6 Months and Recognize the Delta Variant. *Clin Infect Dis.* 2021. doi:ciab915. Data for flow cytometry experiment and ELISpot assays for delta variants were acquired by Caroline Garliss.

6 Chapter 6: COVID-19 mRNA Vaccine-induced T cells recognize the Omicron variant

6.1 Abstract

We compared antibody and T cell responses to the SARS-CoV-2 vaccine strain spike and Omicron variant spike protein in 15 mRNA vaccine recipients. While these individuals had significantly lower levels of antibodies that inhibit Omicron spike protein

binding to ACE2, T cell responses to vaccine strain and omicron variant were comparable.

6.2 Introduction

The Omicron variant was first reported in November 2021 by scientists in South Africa [1]. The variant contains more than 50 mutations including 33 in the spike protein and studies show that this results in evasion of vaccine-elicited neutralizing antibodies [2, 3]. However, less is known about how these mutations impact the T cell response to the virus. We compared antibody and T cell responses to the vaccine strain and Omicron variant spike proteins in 15 mRNA vaccine recipients (VRs). Our data may partially explain the clinical outcomes seen in VRs with breakthrough Omicron variant infection.

6.3 Methods

We obtained blood from 15 VRs. Twelve of these VRs received the Pfizer-BioNTech (BNT162b2) vaccine, 3 received the Moderna (mRNA-1273) vaccine, 13 received a booster vaccine. The median age of the participants was X years (range: 23 to 56 years). Informed consent was obtained from all study participants.

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll centrifugation. Plasm antibody responses were measured using the Meso Scale Discoveries pseudoneutralization/ACE2 inhibition assay (Rockville, Maryland, USA), which measures the ability of participant plasma to inhibit ACE2 binding to spike proteins from the vaccine strain and multiple VOCs. The levels of inhibiting antibody measured with this assay correlate well with culture-based neutralization assays [4]. The assay was performed with plasma diluted at 1:100 as previously described [4].

We determined cellular immunity to the SARS-CoV-2 spike protein by performing an interferon-γ (IFN-γ) Elispot assay with unfractionated PBMCs as previously described [5]. The assay was also performed with CD8+ T-cell–depleted PBMCs to determine the relative contribution of CD4+ T cells and CD8+ T cells to the cellular immune response. To compare recognition of spike proteins from the vaccine strain and the Omicron variant, we stimulated PBMCs with overlapping spike peptide pools from both viruses at a concentration of 1 μg/mL (JPT, Berlin, Germany). Both spike peptide pools were made up of of 315 peptides that were mostly 15 amino acids long with an overlap of 11 amino acids. T cell responses to overlapping peptide pools of nucleocapsid protein at 10ug/ml (BEI, Manassas, VA) using INF-γ ELISpot were also measured to rule out prior natural infections.

Statistical comparisons were done using GraphPad Prism 9.2.0. Comparisons were made with One-way ANOVA with Geisser-Greenhouse correction Dunnet's multiple comparison test was done, with individual variances computed for each comparison. P value < 0.05 was considered significant

6.4 Results

The level of antibodies in VR plasma that inhibited the binding of ACE2 to spike proteins from the Alpha, Beta, Delta and Omicron variants were significantly lower than the level of antibodies that inhibited the binding of ACE2 to the vaccine strain spike protein. ACE2 binding to the Omicron spike protein was least inhibited by the VR plasma (Figure 6.1A).

In contrast, the VRs made robust T cell responses to peptide pools from both vaccine strain and Omicron spike proteins (Figure 6.1B). Interestingly, depletion of CD8+ T cells

did not result in a significant decrease in the total T cell response to both sets of spike peptides implying that CD4+ T cells were the major producers of IFN-y in the assay (Figure 6.1C). There was a strong correlation between T cell responses to spike peptides from the vaccine strain and the omicron variant with both unfractionated (Figure 6.1D) and CD8 depleted T cells (Figure 6.1E) suggesting that there was cross recognition of the epitopes in the 2 proteins. None of the VRs had T cell responses to nucleocapsid peptides suggesting that there were no cases of asymptomatic infection.

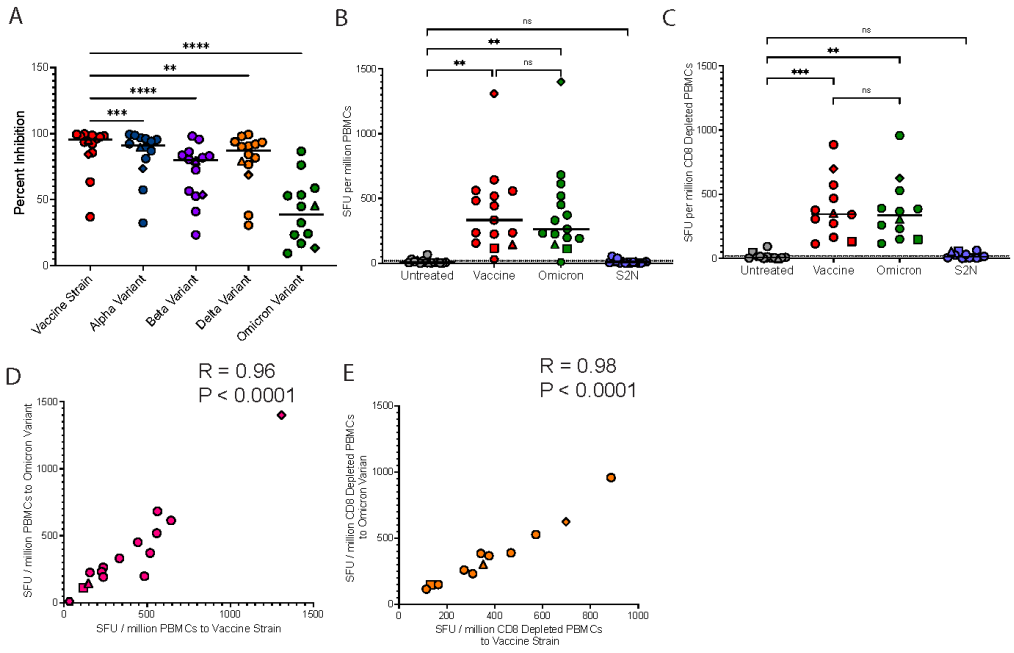

Figure 6.1:Antibody and T cell responses to vaccine strain and Omicron variant spike proteins. The level of antibodies that inhibit ACE2 binding to spike are shown for the vaccine strain, and different variants of concern (A). The number of SFU per million cells generated in response to stimulation with vaccine strain or Omicron variant spike peptides or nucleocapsid peptides (S2N) is shown for unfractionated PBMCs (B) and CD8-depleted PBMCs (C). Horizontal bars represent the median value. The frequency of SFU per million cells generated in response to stimulation with the vaccine strain spike peptides is compared to the frequency of SFU per million cells generated in response to Omicron variant spike peptides for PBMCs (D) and CD8-depleted PBMCs (E). 0.1234 (ns), 0.0332 (∗), 0.0021 (∗∗), 0.0002 (∗∗∗), <0.0001 (∗∗∗∗). Abbreviations: IFN-γ, interferon-γ; ns, nonsignificant; PBMC, peripheral blood mononuclear cell; SFU, spot forming units.

6.5 Discussion

In this study we compared antibody and T cell responses in mRNA vaccine recipients. We found lower levels of antibodies that inhibited the binding of ACE2 to the Omicron spike protein consistent with prior studies [2, 3]. In contrast, T cells from the vaccine recipients recognized overlapping peptides from both proteins. Studies that have analyzed the epitopes targeted by T cells from patients with natural infection have concluded that the breadth of the response makes escape by a variant unlikely [6]. Furthermore, mRNA vaccine-elicited T cells have been shown to recognize prior SARS-CoV-2 variants [5, 7]. Gao et al recently reported T cell cross-recognition of Omicron spike peptides by convalescent COVID-19 patients and mRNA vaccine recipients [8]. Other recent studies have reported similar findings [9- 11]. We confirm this T cell mediated recognition of the Omicron variant and extend the findings by directly comparing these responses to functional antibody responses in the same individuals. The ability of the Omicron variant to evade antibody responses may explain why breakthrough infections are seen even in boosted vaccine recipients [12]. However, the strong T cell responses seen in vaccine recipients may provide protection against severe disease in these individuals. The correlation between T cell responses to the vaccine strain and Omicron variant spike proteins is likely due to cross recognition of epitopes in spite of the large number of mutations present in the Omicron variant. This lack of significant T cell escape by Omicron and prior variants may suggest that mRNA vaccine-elicited T cell responses may be effective against future variants of concern that evade antibody responses.

6.6 References

1. WHO. Classification of omicron (B.1.1.529): SARS-CoV-2 variant of concern. Statement
   26 November, 2021. https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern
2. Lu L, Mok BW, Chen LL, et al. Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or Coronavac vaccine recipients. Clin Infect Dis. 2021 Dec 16:ciab1041.
3. Nemet I, et al. Third BNT162b2 Vaccination Neutralization of SARS-CoV-2 Omicron Infection. N Engl J Med. 2021 Dec 29.
4. Karaba AH, Zhu X, Liang T, et al. A third dose of SARS-CoV-2 vaccine increases neutralizing antibodies against variants of concern in solid organ transplant recipients. Am J Transplant. 2021 Dec 24. doi: 10.1111/ajt.16933. Epub ahead of print. PMID: 34951746.
5. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. J Clin Invest. 2021 May 17;131(10):e149335.
6. Redd AD, Nardin A, Kared H, et al. CD8+ T-Cell Responses in COVID-19 Convalescent Individuals Target Conserved Epitopes From Multiple Prominent SARS-CoV-2 Circulating Variants. Open Forum Infect Dis. 2021 Mar 30;8(7):ofab143.

7. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4+ and CD8+ T cell reactivity in infected or vaccinated individuals. Cell Rep Med. 2021 Jul 20;2(7):100355.
8. Gao Y, Cai C, Grifoni A, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. Nat Med. 2022 Jan 14.
9. Keeton R, Tincho MB, Ngomti A, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. Nature. 2022 Jan 31.
10. Liu J, Chandrashekar A, Sellers D, et al. Vaccines Elicit Highly Conserved Cellular Immunity to SARS-CoV-2 Omicron. Nature. 2022 Jan 31.
11. Tarke A, Coehlo C, Zhang, Z et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell. 2022 Jan 23
12. Kuhlmann C, Mayer CK, Claassen M, et al. Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. Lancet. 2022 Jan 18:S0140-6736(22)00090-3.

Contributions and Acknowledgments:

Meso scale discovery ACE2 inhibiting assays were acquired by Dr. Andrea Cox's group and Dr. Andrew Karaba's group. ELISpot assays were done in conjunction with Caroline Garliss.

7 Chapter 7: SARS-CoV-2-specific immune responses in boosted vaccine recipients with breakthrough infections during the Omicron variant surge

7.1 Abstract

Breakthrough SARS-CoV-2 infections in vaccinated individuals have been previously associated with suboptimal humoral immunity. However, less is known about breakthrough infections with the Omicron variant. We analyzed SARS-CoV-2 specific antibody and cellular responses in healthy vaccine recipients who experienced breakthrough infections a median of 50 days after receiving a booster mRNA vaccine with an ACE2 binding inhibition assay and an ELISpot assay respectively.

We found high levels of antibodies that inhibited vaccine strain spike protein binding to ACE2 but lower levels that inhibited Omicron variant spike protein binding to ACE2 in four boosted vaccine recipients prior to infection. The levels of antibodies that inhibited vaccine strain and Omicron spike protein binding after breakthrough in 18 boosted vaccine recipients were similar to levels seen in COVID-19 negative boosted vaccine recipients. In contrast, boosted vaccine recipients had significantly stronger T cells responses to both vaccine strain and Omicron variant spike proteins at the time of breakthrough. Our data suggest that breakthrough infections with the Omicron variant can occur despite robust immune responses to the vaccine strain spike protein.

7.2 Introduction

The Omicron variant of concern (B.1.1.529) was identified in November 2021 in South Africa and has since spread across the globe replacing the Delta variant as the dominant strain (1). Omicron has over 50 mutations in its genome, with over 30 mutations residing in the spike protein (1). There is evidence that Omicron is more transmissible (1) and infectious (2) than previous variants of concern (VOCs). Moreover, Omicron effectively evades vaccine-elicited neutralizing antibodies, with two doses of COVID-19

mRNA vaccine inducing minimal antibody responses that can cross-neutralize Omicron (3-8). Booster doses enhance levels of omicron neutralizing antibodies, however, these responses remain 4 to 6 times lower than responses to vaccine strain spike protein (3-6). Unlike neutralizing antibodies, vaccine-induced T cell responses can cross-recognize the omicron spike protein (9-15) and this may partially explain protection against severe disease.

COVID-19 mRNA vaccines have strong efficacy against prior VOCs including the Delta variant, however the efficacy is much lower against the Omicron variant after a two-dose COVID-19 mRNA vaccination regimen (16-19). One study found that vaccine efficacy against the Omicron variant infection was 44% at 14-90 days following the second dose, and declined dramatically over time (16). A second study found vaccine effectiveness against symptomatic infection after two BNT162b2 doses was 65.5% at 2 to 4 weeks, but dropped to 8.8% after 25 weeks (19).

A third vaccine dose increases protection from all VOCs, however efficacy against the Omicron variant remains much lower compared to the Delta variant and declines over time. Andrews et al. reported that vaccine effectiveness against symptomatic Omicron variant infection increased to 67.2% at 2 to 4 weeks after a BNT162b2 booster dose before declining to 45.7% at 10 weeks (19). In another study, Tseng et al showed that vaccine effectiveness against infection 2 months after a booster dose was 86% against the Delta variant and 47% against the Omicron variant (16).

Breakthrough infections with the Alpha variant in fully vaccinated individuals have been associated with lower titers of neutralizing antibodies (20-22) and less robust T cell

responses (23). However, given that the Omicron variant has more mutations and evades neutralizing antibody responses better than prior VOCs, the mechanisms of Omicron variant breakthrough infections are likely different. Thus, it is important to analyze immune responses prior to and following Omicron variant breakthrough infections in fully vaccinated as well as boosted individuals.

In this study, we determined antibody and T cells responses following breakthrough infections in 18 boosted VRs during the Omicron variant surge. Importantly, we were able to study immune responses in four VRs prior to breakthrough infections. Our data advance our understanding of breakthrough infections in vaccinated individuals.

7.3 Methods

Study participants.

Breakthrough vaccine recipients (VRs) refers to study participants who experienced breakthrough infections after full vaccination followed by an additional booster dose. The 18 breakthrough VRs had no comorbidities and a mean age of 30 years (range 23 to 62 years). 14 of these VRs were female and 4 were male. Informed consent was obtained from all participants. COVID-19 diagnosis was made by PCR on sputum or nasal swab specimens in 14 participants and by an antigen test in 4 participants. All infected participants experienced mainly mild upper respiratory tract symptoms. Fourteen of the breakthrough VRs received three doses of the BNT162b2 (Pfizer) vaccine, three received an initial dose of the Ad26.COV2 (Johnson & Johnson) vaccine followed by a booster dose of the mRNA1273 (Moderna) vaccine and one received the three doses of the mRNA1273 vaccine (Table 7.1). The median time between the

receipt of the booster dose and the onset of symptoms was 50 days (range 14 to 92 days) for all 18 VRs. The median time between symptom onset and sampling for immune response analysis was 11 days (range 6 to 19 days). As part of a separate analysis, we also obtained samples from 5 boosted VRs at early (median 3 days after the onset of symptoms, range 1 to 4 days) and later (median 8 days, range 6 to 10) time points. Furthermore, we tested pre-infection immune responses in 4 boosted VRs (VR21, 26, 37, 97) between 5 to 21 days after the booster vaccine dose. The median time to symptom onset for these four subjects was 45 days (range 32 to 76). More information on breakthrough VRs is presented in Table 7.1.

Breakthrough VRs were compared to boosted study participants with no prior history of COVID-19 infections (termed post-boost VRs). For post-boost VRs, the first cohort was sampled 1-3 weeks following booster shots (n=31). 15/31 of these participants were female. 28 participants received three doses of BNT162b2 vaccine, 1 received 3 doses of mRNA1273 vaccine, 2 received two doses of BNT162b2 vaccine followed by a mRNA1273 booster dose. Age of study participants ranged from 21-60, with 10 participants being 21-30, 7 participants being 21-40, 7 participants being 41-50 and remaining 7 participants being 51-60.

A second cohort of post-boost VRs were samples 1-3 months following booster doses (n=13) and had no prior history of COVID-19 infections. 7/13 were female. 11 received three doses BNT162b2 vaccinations, 1 received three doses of mRNA1273, and 1 received two doses of BNT162b2 followed by an mRNA1273 booster shot. Age ranged from 21-60, with 7 participants age 21-30, 2 participants being 31-40, 2 participants being 41-50, and 2 being 51-60.

A third cohort of post-boost VRs were sampled 1-4 weeks following booster doses and were used for T cell responses comparisons for the Omicron variant (n=11). 6 out of 11 were female. All participants received three doses of BNT162b2. Age ranged from 21-60, with 4 being 21-30, 2 being 31-40, 4 being 41-50 and 1 being 51-60.

A cohort of VRs who received full doses of vaccination with no prior history of COVID-19 infections and sampled greater than 6 months following their second dose of vaccination (termed pre-boost VRs, n=21) were also used for comparisons. 10 out of 21 donors were female. 20 received two doses of BNT162b2 and 1 received mRNA1273. Age ranged from 21-60, with 7 being 21-30, 5 being 31-40, 5 being 41-50 and 4 being 51-60.

Spike-binding antibody assay.

The Euroimmun Anti-SARS-CoV-2 immunoglobulin G (IgG) ELISA assay (MountainLakes, New Jersey, USA) was used to measure the titer of binding antibody to the vaccine-strain spike protein as previously described (25). Antibodies to the nucleocapsid protein were measured with the Bio-Rad Platelia SARS-CoV-2 Total Ab assay (Marnes-la-Coquette, France) and used to rule out asymptomatic infection. Seven VRs had positive responses at the time of breakthrough and two others had indeterminate responses (Figure 7.6C). Interestingly, 16 of 17 breakthrough VRs had detectable T cell responses to the nucleocapsid peptide pool at this time point (Figure 7.6D).

Receptor Binding Domain (RBD) Binding Assay.

Antibodies against the receptor binding domain (RBD) of SARS-CoV-2 were measured in plasma using the Meso Scale Diagnostics (MSD, Rockville, MD) Coronavirus Panel 3

IgG kit at a dilution of 1:5000 according to the manufacture's protocol. This is an electrochemoluminescent sandwich ELISA-based assay used in multiple studies of SARS-CoV-2 antibodies (26). Each sample was measured in duplicate. Plates were read on a MESO QuickPlex SQ 120 and arbitrary units (AU) were calculated using the MSD Discovery Workbench software according to the manufacturer's protocol. Conversion to WHO binding antibody units (BAU) were performed by multiplying AU by the manufacturer's verified conversion factor. Seropositivity cutoffs for SARS-CoV-2 specific antibodies were provided by the manufacturer and are based on convalescent samples. Data is presented on a log scale.

ACE2/Spike inhibition assay.

The Meso Scale Discovery (MSD) ACE2 inhibition assay measures the ability of plasma to inhibit ACE2 binding to full-length spike protein, a surrogate measure of neutralization). Previous data have indicated that a cutoff of 20% ACE2 inhibition is associated with measurable live virus neutralizing antibody, including versus variants of concern (26). Briefly, plasma from study participants was thawed and ACE2 inhibition was measured using the ACE2 MSD V-PLEX SARS-CoV-2 23 kits according to the manufacturers' protocol at a dilution of 1:100. Specifically, plates were pre-coated by the manufacturer with spike proteins corresponding to variants of interest (i.e., expressing key mutations). The plates were washed and incubated with plasma for one hour followed by the addition of human ACE2 protein conjugated with a SULFO-TAG (light-emitting label) for another hour. The plates were then washed, read buffer added, and the plates were read with a MESO QuickPlex SQ 120 instrument per the manufacturer's instructions. If the plasma fully bound the coated spike protein and

blocked binding of the added ACE2, then no light was emitted during the electrical stimulation phase of the assay, corresponding to 100% ACE2 inhibition (full surrogate neutralization). Whereas, if there was no effective binding of spike by plasma, then the SULFO-TAG ACE2 fully bound the coated spike protein and illuminated during activation of the chemiluminescent plate, corresponding to 0% inhibition. At least four wells were left blank for calibration to 0% inhibition. Results were reported as percent ACE2 inhibition based on the equation provided by the manufacturer (1 – Average sample ECL/Average ECL signal of blank well) x100.

ELISpot assay.

The interferon-gamma ELISpot assay was used to analyze T cell responses to SARS-CoV-2 spike and nucleocapsid peptide pools (BEI Resources Manassas, VA) and the S1 subunit of the vaccine strain and Omicron variant spike proteins (Genscript Biotech Corporation, Piscataway, NJ). Patient peripheral blood mononuclear cells were incubated with peptides for 20-24 hours or with the spike proteins for 36-40 hours before the plates were developed as previously described (29).

Statistics.

Statistical analyses were performed with Graph Pad Prism in 9.2.0. Statistical tests performed on figure legends. If unpaired, statistical comparisons were done using Ordinary one-way ANOVA and Holm-Sidak's mulitiple comparison test, with a single pool variance used. Paired analyses were done using RM one-way ANOVA with Geisser-Greenhouse corrections and Sidak's multiple comparison test, with individual

variances computed for each comparison. P values < 0.05 were considered significant. *(0.0332), **(0.0021), ***(0.0002), **** (<0.0001).

Study approval.

The study was approved by the IRB of Johns Hopkins University. Written informed consent was obtained from all study participants prior to their inclusion in the study.

Table 7.1: List of vaccine recipients who experienced breakthrough infections after booster shots (breakthrough VRs)

	Vaccine recipient	Days between booster shot and symptom onset	Days between symptom onset and blood draw	Days between booster shot and blood draw	Full vaccine regimen	Booster dose
1	VR6	81	17	98	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
2	VR21	76	6	82	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
3	VR26	60	11	71	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
4	VR37	40	9	49	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
5	VR97	32	7	39	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
6	VR98	64	11	75	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
7	VR99	60	16	76	Ad26.COV2 (Johnson & Johnson)	mRNA1273 (Moderna)
8	VR100	50	7	54	Ad26.COV2 (Johnson & Johnson)	mRNA1273 (Moderna)
9	VR101	66	8	69	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
10	VR102	76	14	90	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
11	VR103	48	19	67	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
12	VR104	14	12	26	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)

13	VR105	83	7	90	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
14	VR106	14	11	25	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
15	VR107	34	10	44	Ad26.COV2 (Johnson & Johnson)	mRNA1273 (Moderna)
16	VR110	92	7	99	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
17	VR111	46	11	57	BNT162b2 (Pfizer)	BNT162b2 (Pfizer)
18	VR113	56	10	66	mRNA1273 (Moderna)	mRNA1273 (Moderna)

7.4 Results

Breakthrough VRs have high levels of vaccine strain spike binding antibodies:

We tested antibody levels in 18 individuals with breakthrough infections who had received a booster mRNA vaccine (referred hereafter as breakthrough VRs). 15 breakthrough VRs received mRNA COVID-19 vaccinations and boosters, and 3 received the Ad26.COV2 (Johnson & Johnson) vaccine followed by an mRNA vaccine booster. The median time between the receipt of the booster dose and the onset of symptoms was 50 days (range 14 to 92 days). The median time between symptom onset and sampling was 11 days (range 6 to 19 days). For four breakthrough VRs (VR21, VR26, VR37 and VR97), we analyzed immune responses between 5 to 21 days after the booster vaccine dose and prior to breakthrough infection. The study design is illustrated in Figure 7.1A and information on the breakthrough VRs is presented in Table 7.1. While we were not able to document infection with the Omicron strain in breakthrough VRs, the participants were infected when this VOC accounted for more than 90% of the SARS-CoV-2 isolates sequenced at the Johns Hopkins Hospital during the 22-day time frame in late December 2021 to mid-January 2022 when the VRs

became symptomatic (24, Figure 7.1B). In addition, we tested immune responses in individuals prior to receiving booster doses more than 6 months following their second mRNA vaccine (referred here after as pre-boost VRs). Furthermore, we tested immune responses in individuals who had no history of COVID-19 and who received booster doses (referred to as post-boost VRs), at either 1-3 weeks or 1-3 months following their booster vaccination.

Figure 1

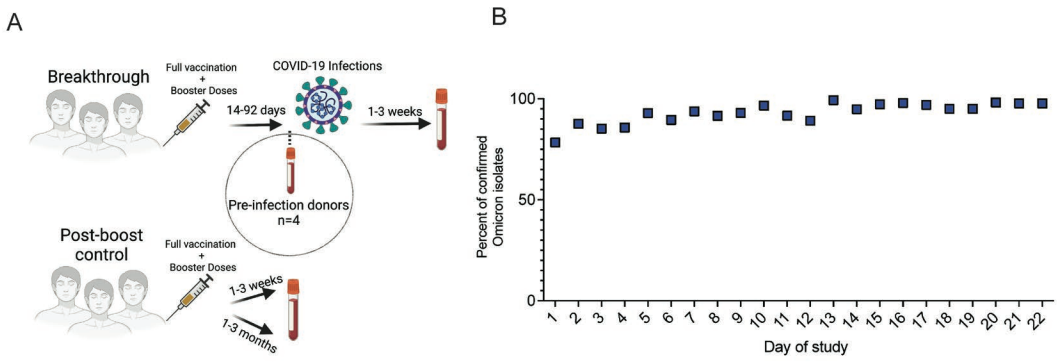

Figure 7.1: Design of observational study of breakthrough boosted vaccine recipients. (A) Observational study design. **(B)** The frequency of Omicron cases among sequenced SARS-CoV-2 isolates at Johns Hopkins Hospital during the study period.

As expected, binding antibodies against vaccine strain spike (Figure 7.2A) and the receptor binding domain (Figure 7.2B) were significantly higher in post-boost VRs compared to pre-boost VRs as measured by the Euroimmun (25) and Meso Scale Discovery binding assays respectively. In the four donors for which we have pre-breakthrough samples (VR21, VR26, VR37 and VR97), antibody levels 1-3 weeks after

boosting were comparable to the post-boost levels seen in post-boost VRs, indicating that these breakthrough VRs had strong peak antibody responses following booster doses (Figure 7.2A, 7.2B). Because post-boost VRs had antibody levels tested 1-3 weeks after the booster shot while breakthrough VRs were tested a median of 67 days after the booster shot (but 1-3 weeks following infection), we also compared the antibody levels from breakthrough VRs to post-boost VRs 1 to 3 months (median of 77 days) after the booster shot (Figure 7.2A). The levels of spike binding antibody were similar to the levels seen at months 1-3 in post-boost VRs with the Euroimmun assay.

Breakthrough VRs have high levels of antibodies that inhibit ACE2 binding to the vaccine strain spike protein:

We then tested antibody-mediated inhibition of spike proteins binding to ACE2 using the Meso Scale Discovery pseudoneutralization/ACE2 inhibition assay, which has been shown to correlate well with a culture-based neutralization assay (26). The degree of ACE2/spike protein binding inhibition was much higher in post-boost VRs than in pre-boost VRs (Figure 7.2C). In post-boost VRs, there was reduced inhibition of ACE2 binding to the spike proteins from the Omicron and Beta variants compared to the vaccine strain, and a very wide range of inhibition of ACE2 binding to the Omicron spike protein. The post-boost plasma from VR21, VR27, VR37, and VR97 strongly inhibited binding of ACE2 to the vaccine strain spike protein. In contrast, inhibition of ACE2 binding to the Omicron spike protein was at the lower end of the spectrum seen with plasma from post-boost VRs (Figure 7.2C). This was also observed to a lesser extent for the Alpha, Beta and Delta variants. Interestingly, plasma samples from breakthrough VRs inhibited binding of ACE2 to all five spike proteins to a degree that was similar to

the level seen with plasma obtained from post-boost VRs at the 1 - 3 month time point, indicating that infection with the Omicron variant did not enhance ACE2 inhibiting antibodies (Figure 7.2D). In order to determine the kinetics of antibody responses, we analyzed ACE2 inhibiting antibodies to different variants in breakthrough VRs 4-7 weeks following symptom onset. We found that antibody levels to all variants tested were slightly higher at this time point but the differences were not statistically significant (Figure 7.2E) and there was no correlation between time after symptom onset and the levels of ACE2 inhibiting antibodies (Figure 7.3).

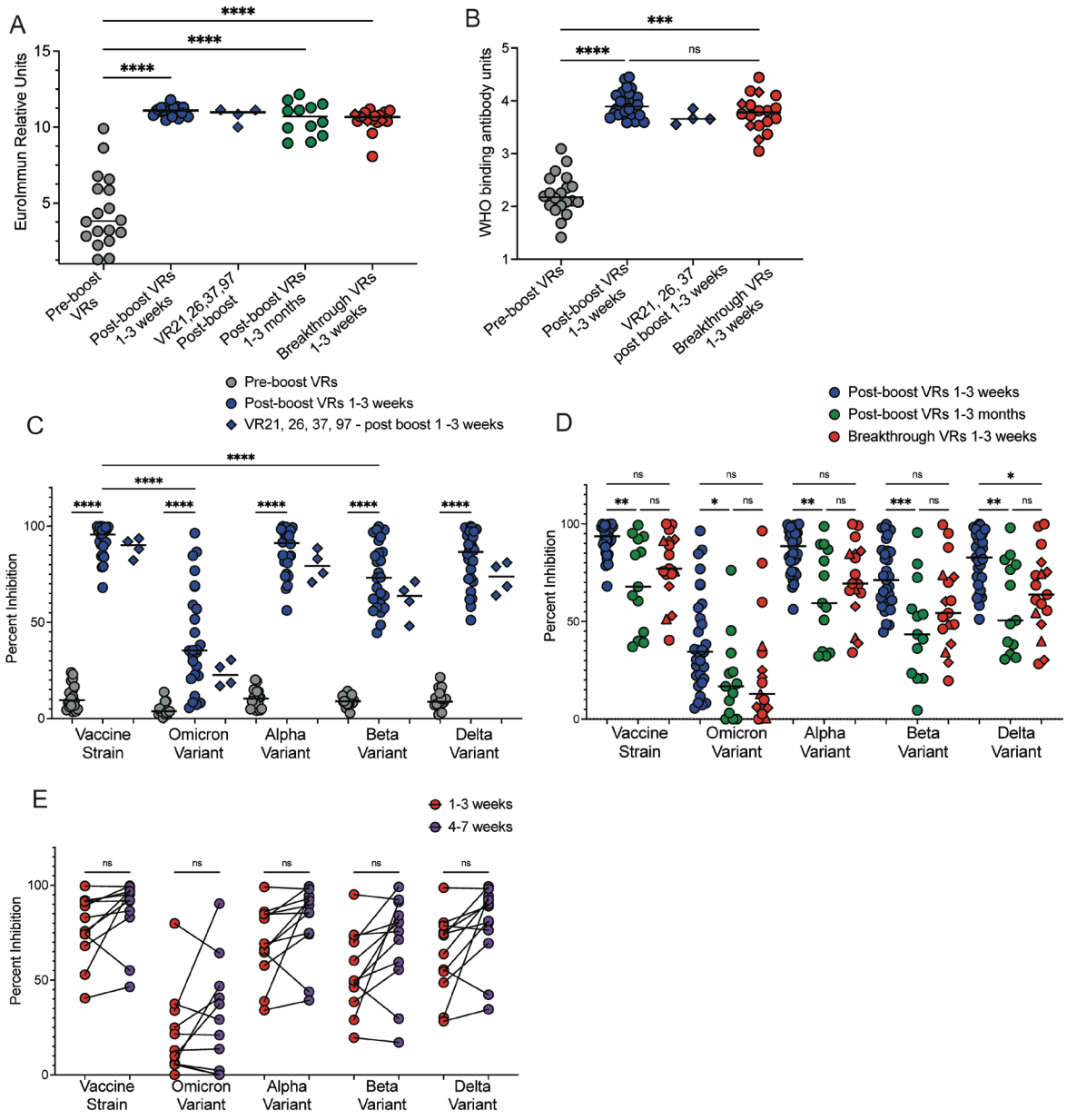

Figure 7.2: Characterization of antibody levels in breakthrough boosted vaccine recipients. (A) Spike binding antibodies found in fully vaccinated individuals prior to their booster shots (Pre-boost VRs), individuals 1-3 weeks (Post-boost VRs 1-3 weeks) or 1-3 months (Post-boost 1-3 months) after their booster shot, breakthrough VRs 1-3 weeks after symptom onset, and VR21, VR26, VR37 and VR 97 1-3 weeks after their

booster shots and before they experienced breakthrough infections. The orange diamonds represent VR 21, 26, 37 and 97 at the breakthrough time point. The orange triangles represent participants who received the Ad26.COV2 vaccine followed by the mRNA1273 booster vaccine. (B) Receptor binding domain (RBD) antibodies in Pre-boost, Post-boost, and Breakthrough VRs as well as VR21, VR26, VR37 at the post-boost time point and prior to infection. The orange diamonds represent VR26 and 37 at the breakthrough time point. Data presented in log scale. (C) Levels of antibodies that inhibit ACE2/spike binding in Pre-Boost and Post-boost VRs and in VR21, VR26, VR37 and VR97 at the 1-3 week post-boost time point. (D) Levels of antibodies that inhibit ACE2/spike binding in VRs in Post-boost VRs 1-3 weeks or 1-3 months after their booster shot, and breakthrough VRs 1-3 weeks after symptom onset The orange diamonds represent VR21, VR26, VR37, and VR 97. (E) Levels of antibodies that inhibit ACE2/spike binding in paired breakthrough VRs at 1-3 weeks and 4-7 weeks after infection. Statistical comparisons were done using Ordinary one-way ANOVA (unpaired) and Holm-Sidak's mulitiple comparison test, with a single pool variance used (2A-2D). Paired analysis (2E) were done using RM one-way ANOVA with Geisser-Greenhouse

corrections. Sidak's multiple comparison test, with individual variances computed for each comparison. *(0.0332), **(0.0021), ***(0.0002), **** (<0.0001).

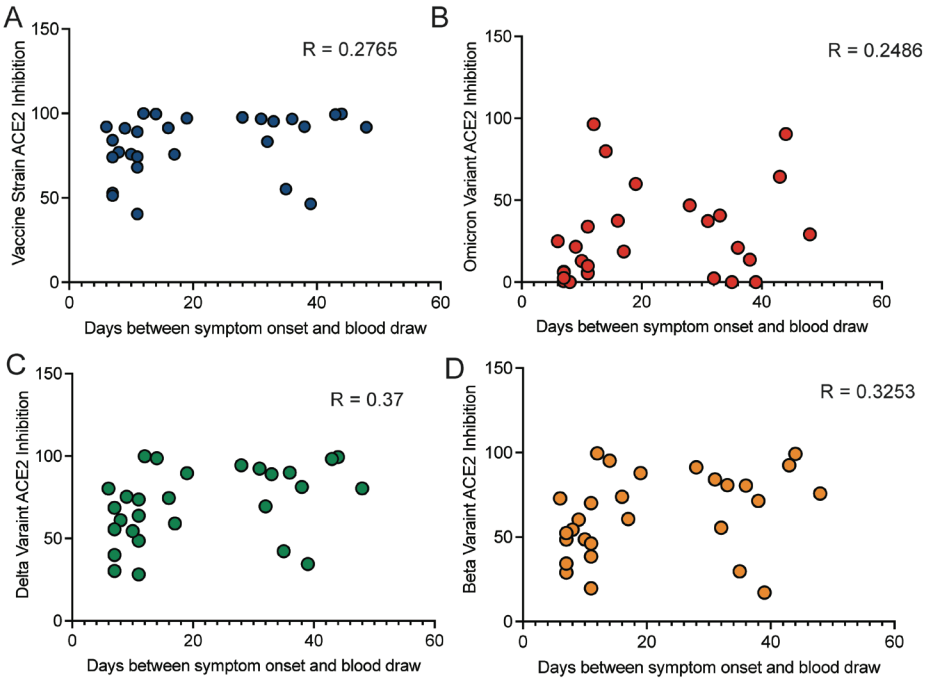

Figure 7.3: Correlation of ACE2 inhibiting antibodies to days since symptom onset. ACE2 inhibiting antibodies to vaccine strain (A), omicron (B), Delta (C) and Beta (D) for breakthrough VRs were correlated with days between symptom onset and blood draw. Pearson correlation coefficients were computed and R values are displayed on each graph.

Breakthrough VRs have robust T cell responses to the vaccine strain and **Omicron variant spike protein:**

We subsequently analyzed T cell responses to the vaccine strain spike protein with the ELISpot assay using overlapping spike peptide pools (27). The number of IFN-y spot

forming units (SFU) measured in VR21, VR26 and VR37 at the post-boost time point was similar to post-boost VRs, indicating strong peak responses following booster doses in these three individuals who eventually had breakthrough infections (Figure 7.4A). Interestingly, PBMCs from the breakthrough VRs generated stronger responses to vaccine strain spike peptides than did PBMCs from post-boost VRs (Figure 7.4A). We also compared responses to the vaccine strain and Omicron variant spike protein S1 subunits and found that the breakthrough VRs made similar responses to the vaccine strain but more potent responses to the Omicron S1 protein than the post-boost VRs (Figure 7.4B), indicating that T cell responses to Omicron variant are likely enhanced by breakthrough infections to this variant.

Since all the post-boost VRs received only mRNA vaccines, we asked whether inclusion of 3 breakthrough VRs who received the Ad26.COV2 vaccine followed by mRNA1273 booster shots affected our results. Exclusion of these 3 breakthrough VRs did not change the results obtained in our antibody and T cell analyses (Figure 7.5).

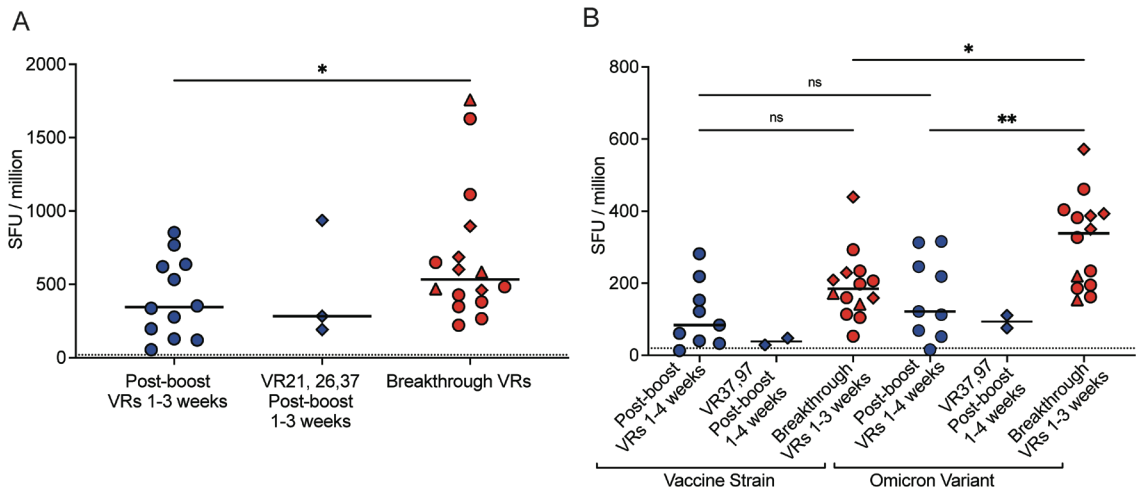

Figure 7.4: Characterization of SARS-CoV-2 specific T cells in breakthrough boosted vaccine recipients. IFN-y ELIspot assay was performed with overlapping spike peptide pools from vaccine strain (A) and S1 spike proteins from vaccine strain or omicron variant (B) in post-boost VRs, in VR21, VR26, and VR37 1-3 weeks post-boost, and in breakthrough VRs. IFN-y spot forming units (SFU) per million PBMCs are shown. Orange diamonds represent VR21, 26, 37 and 97 at the breakthrough time point. Statistical comparisons were done using Ordinary ony way ANOVA (unpaired) and Holm-Sidak's mulitiple comparison test, with a single pool variance used. *(0.0332), **(0.0021).

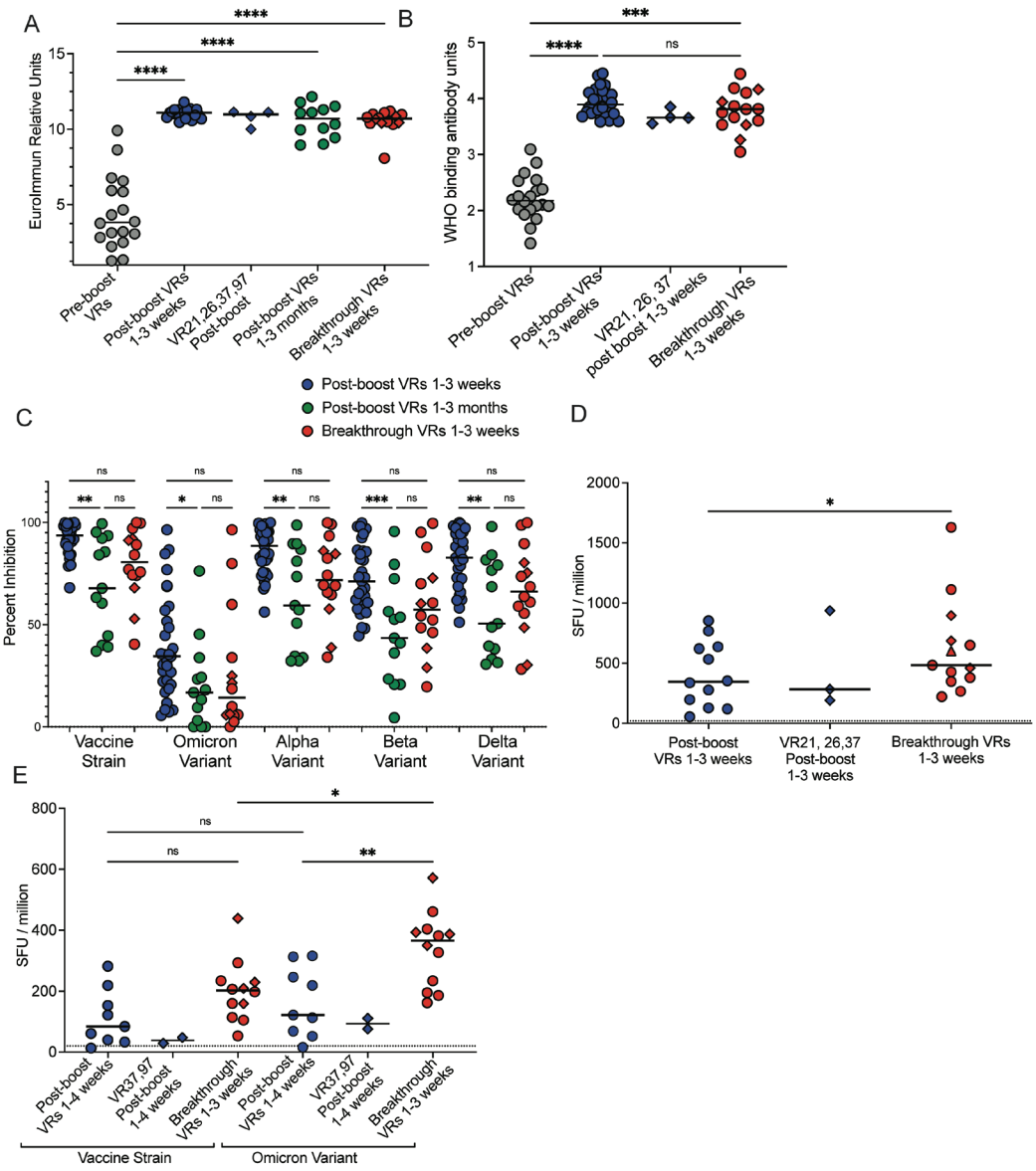

Figure 7.5: Characterization of immune responses following breakthrough infections in breakthrough VRs who only received mRNA vaccinations. Data from Figure 2 and 3 are displayed with breakthrough donors receiving Ad26.COV2 (Johnson & Johnson) excluded. (A) Spike binding antibodies found in mRNA vaccinated individuals prior to their booster shots (Pre-boost VRs), individuals 1-3 weeks (Post-boost VRs 1-3 weeks) or 1-3 months (Post-boost 1-3 months) after their booster shot,

breakthrough VRs 1-3 weeks after symptom onset, and VR21, VR26, VR37 and VR 97 1-3 weeks after their booster shots and before they experienced breakthrough infections. The orange diamonds represent VR 21, 26, 37 and 97 at the breakthrough time point. (B) Receptor binding domain (RBD) antibodies in Pre-boost, Post-boost, and Breakthrough VRs as well as VR21, VR26, VR37 at the post-boost time point and prior to infection. The orange diamonds represent VR26 and 37 at the breakthrough time point. (C) Levels of antibodies that inhibit ACE2/spike binding in VRs in Post-boost VRs 1-3 weeks or 1-3 months after their booster shot, and breakthrough VRs 1-3 weeks after symptom onset. The orange diamonds represent VR21, VR26, VR37, and VR 97. (D) IFN-□ ELIspot assay to vaccine strain overlapping spike peptide pools and (E) IFN-y ELIspot assay to S1 protein from vaccine strain or omicron variant in post-boost VRs, in VR21, VR26, and VR37 1-3 weeks post-boost, and in breakthrough VRs. IFN-y spot forming units (SFU) per million PBMCs are shown. Orange diamonds represent VR21, 26, 37 and 97 at the breakthrough time point. Statistical comparisons were done using Ordinary one-way ANOVA (unpaired) and Holm-Sidak's mulitiple comparison test, with a single pool variance used. *(0.0332), **(0.0021), ***(0.0002), **** (<0.0001).

Longitudinal antibody and T cell responses to vaccine strain and Omicron variant spike protein

In the studies described above, we compared responses from breakthrough VRs at a median of 11 days after symptom onset to those from post-boost VRs at a similar time point post vaccination. In order to estimate the contributions of anamnestic responses induced by the breakthrough infection to the total responses seen at the time point most

VRs were studied, we obtained samples from five boosted breakthrough VRs at early (median 3 days after the onset of symptoms, range 1 to 4 days) and later (median 8 days, range 6 to 10 days) time points. We compared inhibition of ACE2 binding to the vaccine strain, Omicron Delta and Omicron spike proteins at the two time points in these 5 VRs (Figure 7.6 A-C) and found no significant increase in the level of inhibiting antibodies. We also found no significant difference in the T cell response to the vaccine strain spike peptides in this time frame (Figure 7.6D). In order to determine change in antibody levels after of breakthrough, we compared levels 1-3 weeks and 1-3 months after breakthrough to levels found 1-3 weeks after boosting in VR21, VR26, VR37, and VR97. There was no appreciable decay in the levels of spike binding antibodies (Figure 7.7A). However, there was a modest decay in the inhibition of ACE2 binding to all five spike proteins in VR21, VR26, VR37, and VR97 between the post-boost and 1-3 week breakthrough time point followed by a subsequent increase at the 4-7 week time point (Figure 7.7B).

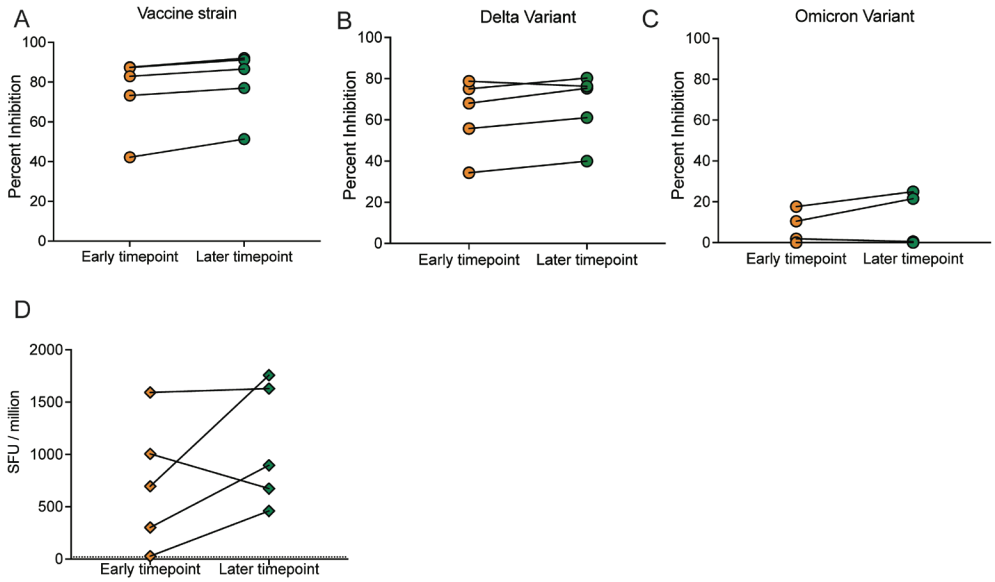

Figure 7.6: Characterization of longitudinal SARS-CoV-2-specific antibody and T cell responses in breakthrough boosted vaccine recipients. Levels of antibodies that inhibit ACE2/spike binding for the vaccine strain (A), and Delta (B) and Omicron (C) variants in 5 Breakthrough VRs at either an early (days 1-4) or later (days 4 to 10) time point. (D) T cell responses measured as IFN-y spot forming units (SFU) per million PBMCS to vaccine strain peptides at an early or later time point.

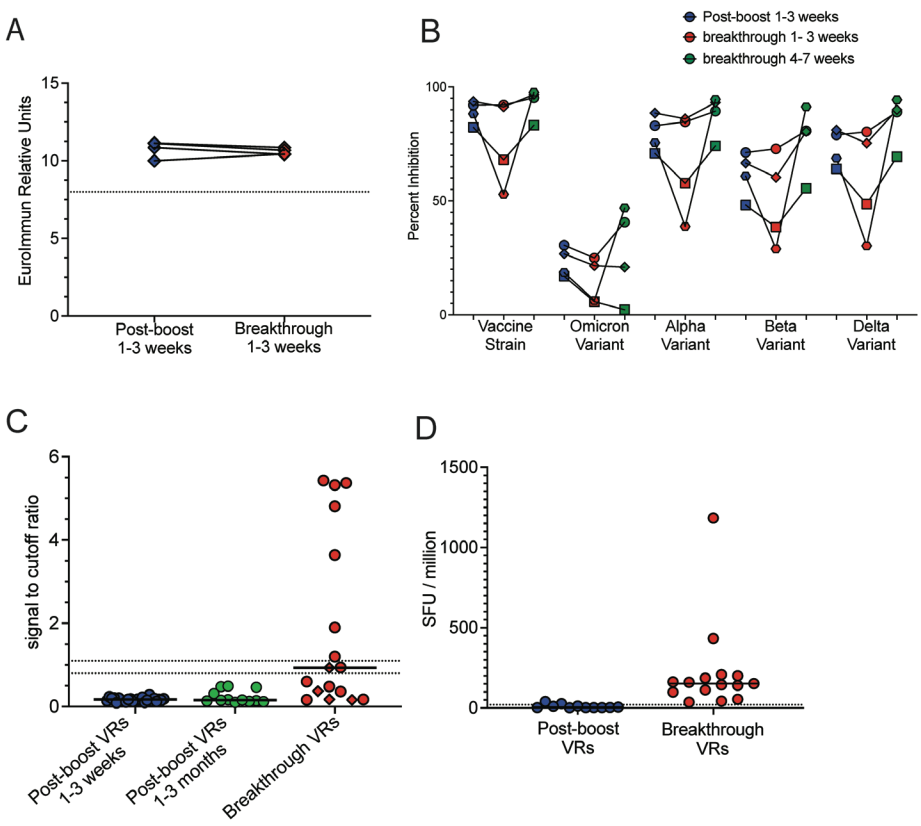

Figure 7.7: Antibody and T cell responses to spike and nucleocapsid proteins. (A) Spike binding antibodies present in VR21, VR26, VR37 and VR97 after booster shots and after breakthrough infection. (B) Levels of antibodies inhibiting ACE2/spike binding with the vaccine strain and with variants of concern in VR21, VR26, VR37 and VR97 after booster shots and after breakthrough infection. (C) Antibody responses to the nucleocapsid protein in post-boost VRs and breakthrough VRs. The lower line represents cutoff for negative responses and the upper line denotes the cutoff for indeterminate responses. (D) T cell responses to nucleocapsid peptides in VRs after the booster shot and in VRs with breakthrough infection.

7.5 References

1. Viana R, et al. Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa. Nature. 2022.
2. Syed AM, et al. Omicron mutations enhance infectivity and reduce antibody neutralization of SARS-CoV-2 virus-like particles. medRxiv [Preprint]. 2022 Jan 2:2021.12.20.21268048.
3. Finlayson A, et al. Neutralization of SARS-CoV-2 Omicron by BNT162b2 mRNA vaccine–elicited human sera. Science. 2022;375(6581):678-680.
4. Gruell H, et al. mRNA booster immunization elicits potent neutralizing serum activity against the SARS-CoV-2 Omicron variant. Nat Med. 2022.
5. Planas D, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature. 2022;602(7898):671-675.
6. Garcia-Beltran WF, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell. 2022 Feb 3;185(3):457-466.e4.
7. Lu L, et al. Neutralization of SARS-CoV-2 Omicron variant by sera from BNT162b2 or Coronavac vaccine recipients. Clin Infect Dis. 2021 Dec 16:ciab1041.
8. Cele S, et al. Omicron extensively but incompletely escapes Pfizer BNT162b2 neutralization. Nature. 2021 Dec 23.
9. Tarke A et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. Cell. 2022

10. Gao Y, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. Nat Med. 2022 Jan 14.
11. Keeton R, et al. T cell responses to SARS-CoV-2 spike cross-recognize Omicron. Nature. 2022 Jan 31.
12. Liu J, et al. Vaccines Elicit Highly Conserved Cellular Immunity to SARS-CoV-2 Omicron. Nature. 2022 Jan 31.
13. Naranbhai V, et al. T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. Cell. 2022 Feb 3:S0092-8674(22)00140-4.
14. GeurtsvanKessel CH, et al. Divergent SARS CoV-2 Omicron-reactive T- and B cell responses in COVID-19 vaccine recipients. Sci Immunol. 2022 Feb 3:eabo2202.
15. Redd AD, et al. Minimal Crossover between Mutations Associated with Omicron Variant of SARS-CoV-2 and CD8+ T-Cell Epitopes Identified in COVID-19 Convalescent Individuals. mBio. 2022 Mar 1:e0361721. doi: 10.1128/mbio.03617-21. Epub ahead of print. PMID: 35229637.
16. Tseng HF, et al. Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. Nat Med. 2022.
17. Collie S, et al. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. N Engl J Med. 2022;386(5):494-496.
18. Lauring AS, et al. Clinical Severity and mRNA Vaccine Effectiveness for Omicron, Delta, and Alpha SARS-CoV-2 Variants in the United States: A Prospective Observational Study. medRxiv. 2022:2022.02.06.22270558.

19. Andrews N, et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. N Engl J Med. 2022.
20. Bergwerk M, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. N Engl J Med. 2021 Oct 14;385(16):1474-1484.
21. Rovida F, et al. SARS-CoV-2 vaccine breakthrough infections with the alpha variant are asymptomatic or mildly symptomatic among health care workers. Nat Commun. 2021 Oct 15;12(1):6032. doi: 10.1038/s41467-021-26154-6. PMID: 34654808; PMCID: PMC8521593.
22. Park HS, et al. Adaptive immune responses in vaccinated patients with symptomatic SARS-CoV-2 Alpha infection. JCI Insight. 2022 Feb 1:e155944.
23. Paniskaki K, et al. Immune Response in Moderate to Critical Breakthrough COVID-19 Infection After mRNA Vaccination. Front Immunol. 2022 Jan 25;13:816220.
24. Fall A, et al. A quick displacement of the SARS-CoV-2 variant delta with omicron: unprecedented spike in COVID-19 cases associated with fewer admissions and comparable upper respiratory viral loads. medRxiv 2022.01.26.22269927; doi: https://doi.org/10.1101/2022.01.26.22269927
25. Patel EU, et al. Comparative Performance of Five Commercially Available Serologic Assays To Detect Antibodies to SARS-CoV-2 and Identify Individuals with High Neutralizing Titers. J Clin Microbiol. 2021 Jan 21;59(2):e02257-20.
26. Karaba AH, et al. A third dose of SARS-CoV-2 vaccine increases neutralizing antibodies against variants of concern in solid organ transplant recipients. Am J Transplant. 2021 Dec 24.

27. Woldemeskel BA, et al. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. J Clin Invest. 2020 Dec 1;130(12):6631-6638.
28. Dimeglio C, et al. Antibody titers and breakthrough infections with Omicron SARS-CoV-2. J Infect. 2022 Feb 3:S0163-4453(22)00060-3.
29. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. J Clin Invest. 2021 May17;131(10):e149335.

Contributions and Acknowledgments:

This chapter has been accepted for publication at JCI insight (March 2022) and should be published with minor edits. Euroimmune binding antibody assays and Biorad nucleocapsid ELISA assays were acquired by Dr. Oliver Laeyendecker's group. Meso scale discovery ACE2 inhibiting and binding assays were acquired by Dr. Andrea Cox's group and Dr. Andrew Karaba's group. ELISpot data were acquired in conjunction with Caroline Garliss.

8 Chapter 8: The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living With Human Immunodeficiency Virus (HIV)

8.1 Introduction

The BNT162b2 messenger RNA (mRNA) vaccine induces robust and protective humoral and cellular response to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein [1] and provides protection from infection with SARS-CoV-2 [2]. However, prior studies have shown suboptimal responses to some vaccines in

people living with human immunodeficiency virus (HIV, PLWH) [3]. A recent study demonstrated that the ChAdOx1 nCoV-19 (AZD1222) vaccine was effective at inducing humoral and cellular immune responses in PLWH [4], but few studies have addressed the immunogenicity of mRNA vaccines in these patients [5, 6]. Here we determined the capacity of the BNT162b2 mRNA vaccine to induce effective cellular and humoral immune responses in PLWH.

8.2 Methods

We obtained blood between 7 and 17 days after the second vaccine dose from 12 PLWH (7 women, 5 men) and 17 healthy donors (7 women, 10 men). None of these individuals had evidence of prior SARS-CoV-2 infection by history or by serology as described below. Informed consent was obtained from all study participants. All PLWH were on antiretroviral therapy (ART) and had a median CD4 + T cell count of 913 cells/uL (range of 649 to 1678 cells/uL). Eleven of the 12 PLWH were African American. Three participants had low level viremia de-spite being on ART (Supplementary Table 8.1). Peripheral blood mononuclear cells (PBMCs) and plasma were isolated from whole blood using ficoll centrifugation. We determined cellular immunity to the SARS-CoV-2 spike protein by performing an interferon-gamma (IFN-γ) Elispot assay with unfractionated PBMCs that were stimulated with a pool of overlapping SARS-CoV-2 spike peptides as previously described [7]. The assay was also performed with CD8 + T cell depleted PBMCs to determine the relative contribution of CD4 + T cells and CD8 + T cells to the cellular immune response. The titer of SARS-CoV-2 spike binding antibodies was determined with the Euroimmun Anti-SARS-CoV-2 immunoglobulin G (IgG) ELISA (Mountain Lakes, New Jersey, USA). Antibodies to the nucleocapsid pro-

tein were measured with the Bio-Rad Platelia SARS-CoV-2 Total Ab assay (Marnes-la-Coquette, France) and used to rule out natural infection with SARS-CoV-2 as mRNA for the nucleocapsid protein is not included in the vaccine. Measurement of antibodies in plasma that block SARS-CoV-2 Spike binding to ACE2 was performed with the MSD V-PLEX SARS-CoV-2 Panel 6 kit from Meso Scale Diagnostics (Rockville, Maryland, USA) using a 1:100 dilution of plasma. Differences in Elispot and Euroimmun values were assessed using a 2-tailed t test. Differences in ACE2 blocking between groups was determined by a 2-tailed Wilcoxon-Mann-Whitney test with a Bonferroni correction, employing R version 4.05. P-values < .05 were considered significant.

8.3 Results

There was no significant difference in titers of SARS-CoV-2 spike binding antibodies in healthy donors (median value of 9.49) and PLWH (median value of 8.84 P = 0.07) (Figure 8.1A). Furthermore, healthy donors and PLWH had similar levels of neutralizing antibodies to the vaccine strain spike protein (Figure 8.1B) and spike proteins from variants of concern (VOC) including the D614G, alpha (B.1.1.7), beta (B.1.351), and gamma (P.1) strains (Figures 8.1B–8.1F). We next compared the cellular responses elicited by overlapping peptides from the vac-cine strain spike protein in PLWH, to responses we obtained in healthy donors from a prior study [7]. There was no significant difference in the number of IFN-γ spot forming units or in the stimulation index (values normalized to media alone) between healthy donors and PLWH in unfractionated PBMCs (Figure 8.1G, 8.1H) or with CD8 + T cell depleted PBMCs (Figure 8.2). Finally, the breadth of the T-cell response was comparable in the 2 groups, and the similar peptide pools were targeted by the 2 study groups (Figure 8.1I).

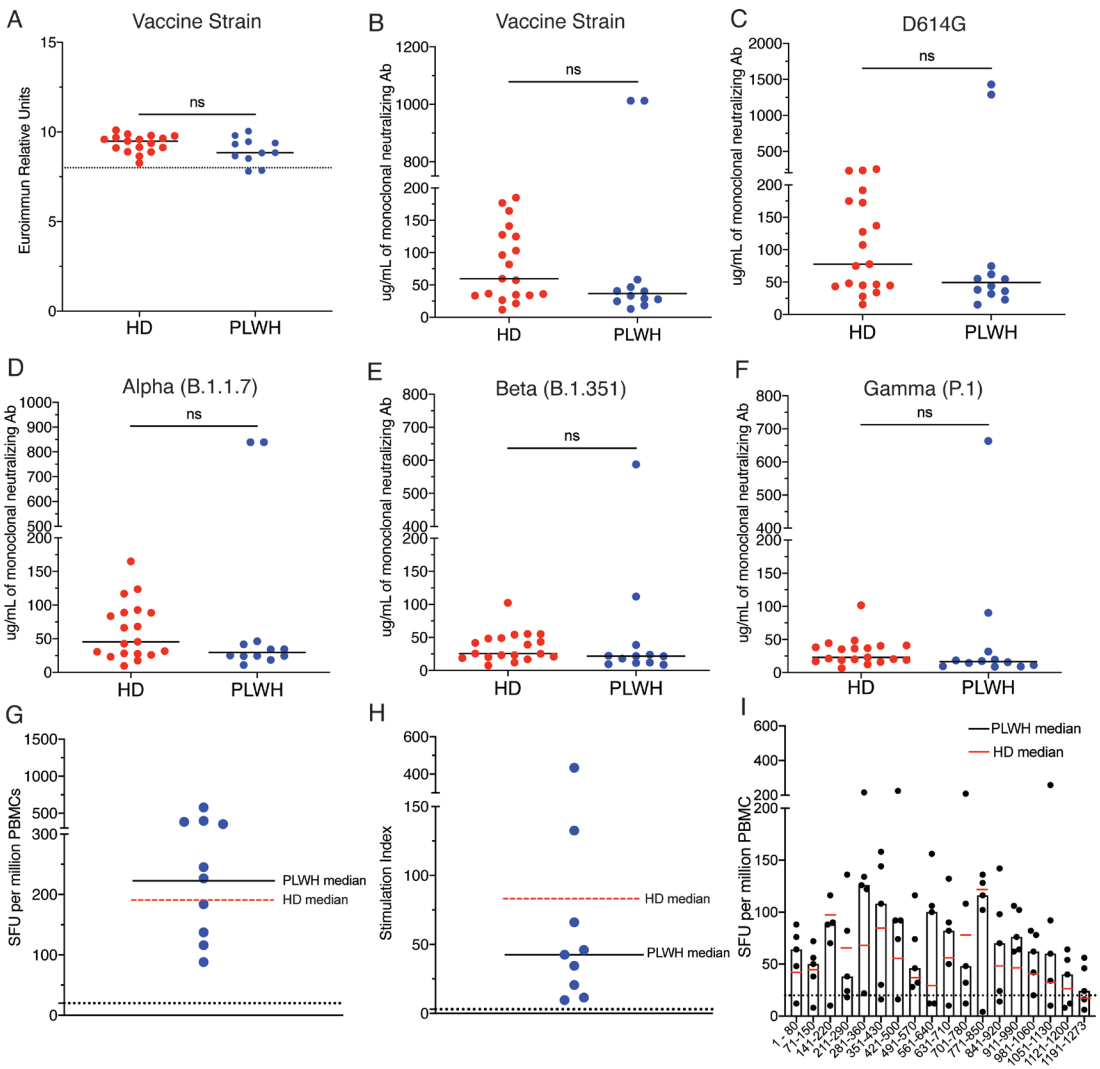

Figure 8.1: Characterization of immune responses in People living with HIV (PLWH) compared to Healthy Donors (HDs). Titer of SARS-CoV-2 spike binding antibodies from HD and PLWH (A). The horizontal line represents the 90th percentile titer in patients with natural infection. Titer of neutralizing antibodies to spike proteins from vaccine strains SARS-CoV2 (B) and variants of concern (C–F). SFUs (G) and SIs (H) to SARS-CoV-2 spike peptide pools from PBMCs from vaccinated PLWH. Black horizontal bars represent the median value for PLWH. Dashed red horizontal bar represents the median value for vaccinated HD from a prior study [7]. Dashed black

horizontal lines denote a significant response (SFU > 20 and SI > 3). Breadth of CD8-depleted T-cell responses from PLWH to pools of 10 peptides that sequentially cover the entire spike protein (E). Horizontal bars represent the median values for PLWH; red horizontal line represents the HD median value. Abbreviations: HD, healthy donors; PBMC, peripheral blood mononuclear cells; PLWH, people living with human immunodeficiency virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot-forming units; SI, stimulation index.

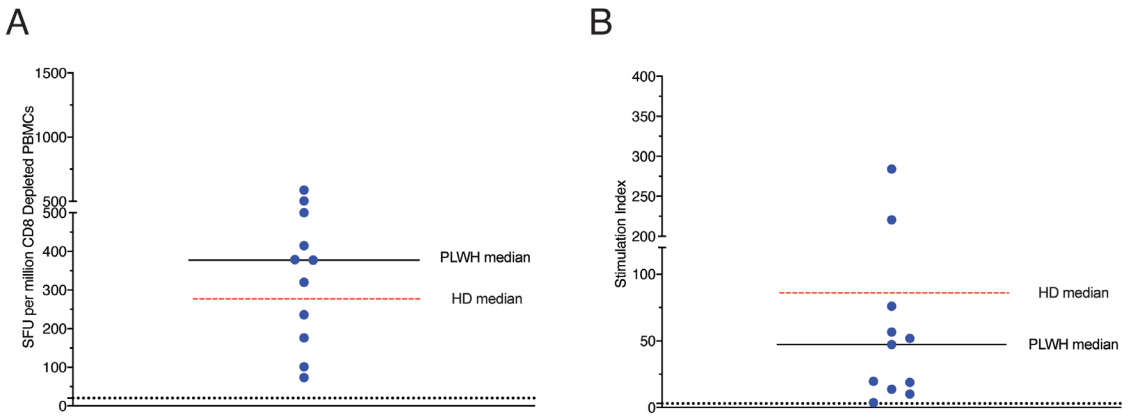

Figure 8.2:CD4 T cell responses in People living with HIV (PLWH) compared to Healthy Donors (HDs. SFUs (G) and SIs (H) to SARS-CoV-2 spike peptide pools from CD8 depleted PBMCs from vaccinated PLWH in INF-y ELISpot assay. Black horizontal bars represent the median value for PLWH. Dashed red horizontal bar represents the median value for vaccinated HD from a prior study [7]. Dashed black horizontal lines denote a significant response (SFU > 20 and SI > 3).

8.4 Discussion

Our study is limited by the relatively small number of participants in both cohorts. Although we screened participants for antibodies to nucleocapsid to rule out prior natural infection, the half-life of antibodies to this protein is relatively short [8]. Thus, we may have missed cases of prior SARS-CoV-2 infection. However, our data confirm a prior study showing that mRNA vaccines induce antibody responses in PLWH [5] and extend the findings by showing that the level of binding antibodies is not significantly different from that produced in healthy donors. These data are similar to results obtained in a phase 2/3 clinical trial in which the ChAdOx1 nCoV-19 (AZD1222) vaccine was shown to elicit strong SARS-CoV-2 specific antibody and T cell responses in PLWH [4]. Of note, in a prior study of naturally infected individuals, antibody titers based on Euroimmun values above 8 were only seen in the top 10% of individuals and were highly correlated with the highest levels of neutralizing titers based on a microneutralization assay [9]. We also demonstrate that neither the magnitude and breadth of vaccine elicited T-cell responses nor the breadth of neutralizing antibodies, as determined by responses to spike proteins from wild-type virus and VOCs, is significantly different between PLWH and healthy donors. These findings are particularly impressive as the PLWH study participants (median age 52 years, range 25–59) were older than the healthy donors (median age 41 years, range 24–59), and the BNT162b2 vaccine induces a lower antibody titer in older individuals [10]. However, this vaccine also elicits a higher antibody titer in women compared to men [10], and our PLWH cohort had a higher frequency of female participants. Of note, prior vaccine studies in PLWH focused mainly [6] or exclusively on men [4]. Data from our balanced cohort strongly suggest that the BNT162b2 vaccine will lead to protection from COVID-19 in

men and women living with HIV. Further studies will be needed to determine whether PLWH with lower CD4 T cell counts have the same robust humoral and cellular responses to the vaccine.

8.5 References

1. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020; 586:594-9.
2. Polack FP, Thomas SJ, Kitchin N, et al; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020; 383:2603–15.
3. Tebas P, Frank I, Lewis M, et al; Center for AIDS Research and Clinical Trials Unit of the University of Pennsylvania. Poor immunogenicity of the H1N1 2009 vaccine in well controlled HIV-infected individuals. AIDS 2010; 24:2187–92.
4. Frater J, Ewer KJ, Ogbe A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in HIV infection: a single-arm substudy of a phase 2/3 clinical trial. Lancet HIV 2021. doi:10.1016/S2352-3018(21)00103-X.
5. Ruddy JA, Boyarsky BJ, Werbel WA, et al. Safety and antibody response to the first dose of SARS-CoV-2 messenger RNA vaccine in persons with HIV. AIDS 2021.
6. Levy I, Wieder-Finesod A, Litchevski V, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in people living with HIV-1. doi:10.2139/ssrn.3829650.

7. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. J Clin Invest 2021; 131:e149335.
8. Lumley SF, Wei J, O'Donnell D, et al. The duration, dynamics and determinants of SARS-CoV-2 antibody responses in individual healthcare workers. Clin Infect Dis 2021; 73:e699–709. doi:10.1093/cid/ciab004.
9. Patel EU, Bloch EM, Clarke W, et al. Comparative performance of five commercially available serologic assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titers. J Clin Microbiol 2021;59:e02257-20.
10. Pellini R, Venuti A, Pimpinelli F, et al. Initial observations on age, gender, BMI and hypertension in antibody responses to SARS-CoV-2 BNT162b2 vaccine. EClinicalMedicine 2021; 36:100928.

9 Chapter 9: Decay of COVID-19 mRNA vaccine-induced immunity in people living with HIV

9.1 Introduction

Current COVID-19 vaccines induce robust humoral and cellular immune responses in healthy vaccine recipients (1) and people living with HIV (PLWH) (2-7). However, while vaccine induced antibody responses wane over time in healthy donors (HDs) (8), it is not known whether PLWH have similar rates of decay of immune responses. Greater declines in antibody titers might explain higher rates of breakthrough infections in PLWH (9) and could explain the increased risk of severe COVID-19 observed in PLWH (10). These studies highlight the importance of understanding vaccine induced immunity in PLWH compared to HDs. We recently

showed that PLWH mount similar antibody and T cell responses following two doses of COVID-19 mRNA vaccinations (7). However, the rate of decay of immune responses in PLWH has not been studied. In this study, we analyzed humoral and cellular immunity at six months post vaccination in PLWH and HDs.

9.2 Methods

The study was approved by the JHU IRB and informed consent was obtained from all study participants. Blood was drawn from 8 PLWH and 25 HDs two weeks and six months after the second dose of COVID-19 vaccinations. 24 HDs received the Pfizer-BioNTech (BNT162b2) vaccine and 1 received Moderna (mRNA-1273) vaccine, while all 8 PLWH received the BNT162b2) vaccine. Age of HDs ranged from 21-60 (7 were 21-30, 5 were 31-40, 5 were 41-50, and 8 were 51-60) and age of PLWH ranged from 41-60 (2 were 41-50, and 6 were 51-60). All PLWH were on suppressive antiretroviral therapy (ART) and had a median CD4+ T cell count of 1044 cells/uL (range of 468 to 1420 cells/uL). Two PLWH had low level viremia (49 and 52 HIV RNA copies /ml respectively), whereas the other six maintained undetectable viral loads (<20 HIV RNA copies /ml) . Antibodies to the nucleocapsid protein were measured with the Bio-Rad Platelia SARS-CoV-2 Total Ab assay (Marnes-la-Coquette, France) and used to rule out natural infection with SARS-CoV-2.

9.3 Results and Discussion

The quantity SARS-CoV-2 spike binding antibodies was determined using Euroimmun Anti-SARS-CoV-2 immunoglobulin G (IgG) ELISA (Mountain Lakes, New

Jersey, USA, 11). PLWH had high levels of binding antibodies at two weeks post second dose that were comparable to peak responses shown in HDs as described in a prior study (7). PLWH had a significant decline in binding antibody responses that was nearly identical to the decline seen in HDs at the six-month time point (Figure 9.1A).

We next compared the level of antibodies in plasma that block ACE2 binding to SARS-CoV-2 spike proteins with the V-PLEX SARS-CoV-2 Panel 13 kit from Meso Scale Diagnostics (Rockville, Maryland, USA, 12). As we have previously reported, at the peak time point, PLWH had a slightly lower but comparable level of inhibiting antibodies to HDs (7). However, at the six-month time point, PLWH had a significantly lower level of ACE2 inhibiting antibodies to the vaccine strain and to the variants of concern (VOC) spike proteins (Figure B-E). The decline in anti-spike antibodies at six months was comparable in PLWH to the decline seen in HDs, for antibodies directed at the spike protein of the vaccine strain (Figure 9.1B) or at the Alpha (Figure 9.1C), the Beta (Figure 9.1D) and Delta (Figure 9.1E) VOC.

We next determined cellular immunity to the SARS-CoV-2 spike protein by performing an interferon-gamma (IFN-γ) ELIspot assay with unfractionated PBMCs that were stimulated with a pool of overlapping SARS-CoV-2 spike peptides (Figure 9.1F). We previously reported that PLWH had robust T cell responses at the peak time point comparable to responses in HDs, (7). Here we show a slight but insignificant decline of these responses at the six-month time point (Figure 9.1F). We then performed the assay using CD8 + T cell depleted PBMCs to determine the relative contribution of CD4 + and CD8 + T cells to the cellular immune response (Figure 9.1G). CD8+ T cell depletion did not decrease the magnitude of the response, suggesting that most of the T cell responses

result from CD4+ T cell responses (Figure 9.1G). We again saw a non-significant decline in CD4+ T cell responses. Collectively, our data suggest that the rate of decay of immune responses in PLWH is similar to healthy donors. Specifically, although antibody responses decline at six months, especially to VOCs spike, T cell responses persist at the six-month time point in PLWH as in HDs (13).

In this study, we show that PLWH have rates of decay of SARS-CoV-2-specific immune responses that are comparable to those of HDs post mRNA SARS-CoV-2 vaccinations. Though our data is limited by the relatively low number of patients in our cohort and further studies will be needed to determine the impact of lower CD4 T cell counts on the vaccine response, we demonstrate that virally suppressed PLWH with high CD4 counts generate robust humoral and cellular immune responses. Our results are consistent with a recent report that showed similar rates of decay of immune responses in PLWH and HD following ChAdOx1 nCoV-19 vaccinations (14).

Our data show that like HDs, PLWH have a significant decline in SARS-CoV-2-specific antibody responses six months following vaccination. These results suggest that PLWH would benefit from an additional booster dose to increase plasma antibody concentrations that could protect against COVID-19. However, recent reports have shown that memory B cell responses persist six months after two doses of vaccination in HDs (15), potentially mitigating the effects of the dramatic decline of antibody responses and consistent with data showing that mRNA vaccines continue to protect against hospitalization six months following vaccination (16). If SARS-CoV-2 specific memory B cells also persist in PLWH, then a similar level of protection from severe disease driven

by memory B cell and effector T cell responses may be seen six months following vaccination.

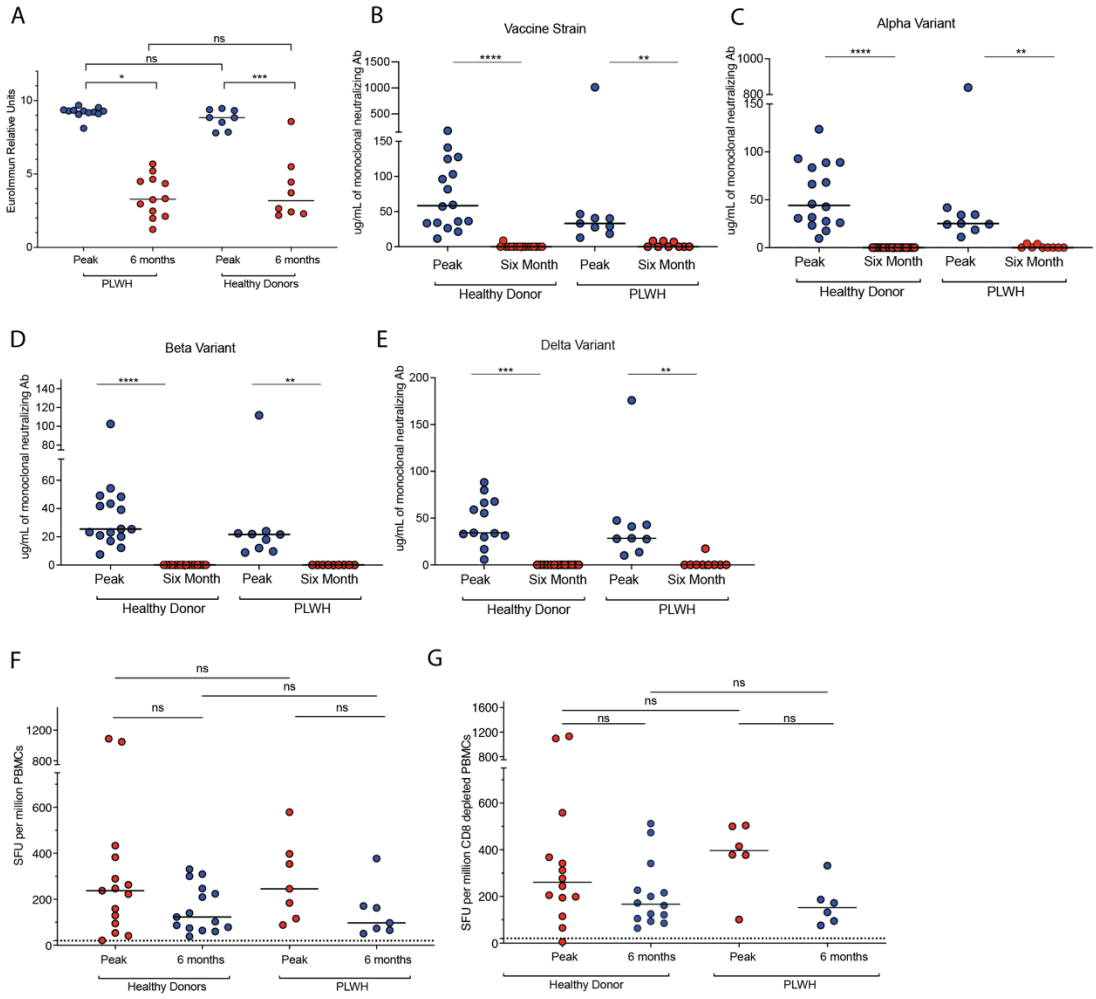

Figure 9.1: Antibody and T cell responses in PLWH and healthy controls at peak and six-months following COVID-19 mRNA vaccinations. PLWH and HD spike binding antibody responses (A) and levels of antibodies that inhibit ACE2 binding to spike proteins of vaccine strain (B), Alpha (C), Beta (D) and Delta (E) viruses. IFN-γ ELISpot

responses to stimulation with spike protein peptide pools in unfractionated PBMCs (F) and CD8 depleted PBMCs (G) in PLWH and HDs. Statistical comparisons for T cell responses were done with Kruskal-Wallis test with Dunn's multiple comparison or Friedman test with Dunn's multiple comparison. Statistical comparisons for Antibody responses were done using Wilcoxon matched-pairs signed rank test or Mann-Whitney test. Abbreviations: HD, healthy donors; PLWH, people living with human immunodeficiency virus; PBMC, peripheral blood mononuclear cells; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot-forming units.

Contributions and Acknowledgments:

This chapter is published in clinical infectious diseases (Woldemeskel BA, Karaba AH, Garliss CC, et al. The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living with HIV. *Clin Infect Dis.* 2021. doi:10.1093/cid/ciab648 [doi].) Euroimmune binding antibody assays and Biorad nucleocapsid ELISA assays were acquired by Dr. Oliver's group. Meso scale discovery ACE2 inhibiting and binding assays were acquired by Dr. Andrea Cox's group and Dr. Oliver Laeyendecker's group. ELISpot data were acquired in conjunction with Caroline Garliss.

9.4 References

1. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 2020; 586:594-9.

2. Madhi SA, Koen AL, Izu A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in people living with and without HIV in South Africa: an interim analysis of a randomised, double-blind, placebo-controlled, phase 1B/2A trial. *The Lancet HIV.* 2021;8(9):e568-e580.
3. Levy I, Wieder-Finesod A, Litchevsky V, et al. Immunogenicity and safety of the BNT162b2 mRNA COVID-19 vaccine in people living with HIV-1. *Clinical Microbiology and Infection.* 2021;27(12):1851-1855.
4. Frater J, Ewer KJ, Ogbe A, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 in HIV infection: a single-arm substudy of a phase 2/3 clinical trial. *The Lancet HIV.* 2021;8(8):e474-e485.
5. Lombardi A, Butta GM, Donnici L, et al. Anti-spike antibodies and neutralising antibody activity in people living with HIV vaccinated with COVID-19 mRNA-1273 vaccine: a prospective single-centre cohort study. *The Lancet Regional Health – Europe.* 2022;13.
6. Rahav G, Lustig Y, Lavee J, et al. BNT162b2 mRNA COVID-19 vaccination in immunocompromised patients: A prospective cohort study. *eClinicalMedicine.* 2021;41.
7. Woldemeskel BA, Karaba AH, Garliss CC, et al. The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living with HIV. *Clin Infect Dis.* 2021.
8. Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N Engl J Med* 2021;385(24):e84.

9. Bhaskaran K, Rentsch CT, MacKenna B, et al. HIV infection and COVID-19 death: a population-based cohort analysis of UK primary care data and linked national death registrations within the OpenSAFELY platform. *The Lancet HIV.* 2021;8(1):e24-e32.
10. Coburn SB, Humes E, Lang R, et al. COVID-19 infections post-vaccination by HIV status in the United States. *medRxiv.* 2021:2021.12.02.21267182
11. Patel EU, Bloch EM, Clarke W, et al. Comparative performance of five commercially available serologic assays to detect antibodies to SARS-CoV-2 and identify individuals with high neutralizing titers. J Clin Microbiol 2021;59:e02257-20.
12. Karaba AH, Zhu X, Liang T, et al. A third dose of SARS-CoV-2 vaccine increases neutralizing antibodies against variants of concern in solid organ transplant recipients. Am J Transplant. 2021 Dec 24. doi: 10.1111/ajt.16933. Epub ahead of print. PMID: 34951746.
13. Woldemeskel BA, Garliss CC, Blankson JN. mRNA Vaccine-Elicited Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)–Specific T Cells Persist at 6 Months and Recognize the Delta Variant. *Clin Infect Dis.* 2021:ciab915.
14. Ogbe A, Pace M, Bittaye M, et al. Durability of ChAdOx1 nCov-19 vaccination in people living with HIV. *JCI Insight.* 2022
15. Goel RR, Painter MM, Apostolidis SA, et al. mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. *Science.* 2021;374(6572):abm0829.

16. Tartof SY, Slezak JM, Fischer H, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *The Lancet.* 2021;398(10309):1407-1416

10 Chapter 10: Concluding Remarks

Since the emergence of the COVD-19 pandemic in early 2019, the scientific community has responded quickly and swiftly to study the virus. In the past two years, the biology, virology and immunology of SARS-CoV-2 and COVID-19 infections have been studied, making SARS-CoV-2 the most studied coronavirus thus far. With remarkable speed, multiple effective vaccines have also been developed and administered worldwide [1]. Despite the tremendous progress made, further work remains to fully understand SARS-CoV-2 and the ongoing COVID-19 pandemic. Critical questions such as the continued evolution of the virus and emerging variants of concern and mechanisms of breakthrough infections following COVID-19 vaccinations remain to be fully characterized. Furthermore, the emergence of future coronavirus pandemics is an imminent threat. Bats are reservoirs for many coronaviruses which might have zoonotic potential [2] and the full extent of the genetic diversity and size of this reservoir is still unknown [3]. Thus, the study of coronaviruses with particular focus on human coronaviruses (both endemic and pandemic causing coronaviruses) are important to inform the development of vaccines and therapies against SARS-CoV-2 and zoonotic coronaviruses. In this section, I will briefly summarize work described in this thesis, and discuss its applications and future directions in the field.

10.1 Summary

In this work, we studied T cell responses to endemic coronaviruses and SARS-CoV-2 following natural infections and vaccination. We show that most individuals have robust T cell responses to common cold coronaviruses, and that T cells have broad responses targeting the entire spike protein of HCoV-NL63. We also look at pre-existing CD4+ T cell responses in health donors and show that pre-existing cells in our donor (HD9) are SARS-CoV-2 / HCoV-NL63 cross-reactive T cells [4]. With this knowledge, and data from Dykema et al. and others that showed cross-reactive T cells in healthy donors and COVID-19 convalescent patients [5], we hypothesized that COVID-19 mRNA vaccinations also enhance responses to CCCs by enhancing cross-reactive T cells, and that responses elicited by vaccinations are broad. Indeed, we show that vaccination enhanced responses to HCoV-NL63, and that vaccines elicit broad T cell responses targeting the entire spike proteome [6]. Additionally, T cells recognize variants of concern (beta, delta and omicron) comparably to the ancestral strain [6,7], because of the breadth of elicited T cell responses. Furthermore, although there was a slight decline, T cell responses are sustained six months after vaccination [7]. Finally, we wondered if vaccinated individuals could target cross-reactive epitopes that are shared among other coronaviruses including bat coronaviruses, and we identified a conserved epitope among human and bat coronaviruses located in the fusion domain that is targeted by cross-reactive T cells [8]. Collectively, our data suggest that T cells elicit robust and broad responses and are more durable than antibody resposnes. Some T cells are also cross-reactive and can recognize other coronaviruses and VOCs. These data highlight the

importance of vaccine-elicited T cell responses and inform the development of T cell-based vaccines, especially in the context of a pan-coronavirus vaccine design.

10.2 Future Directions

It is now seeming increasingly clear that the SARS-CoV-2 will not be eradicated with the current tools in our arsenal. This is more evident with the emergence of the omicron variant and the increased number of breakthrough infections in vaccinated and boosted individuals [9,10], and even in those with strong vaccine-induce immune responses [11]. Thus, the development of second-generation vaccines might be likely to protect against VOCs as well as potentially zoonotic bat coronaviruses. There is an increased call for working towards the development of pan-coronavirus vaccines that can protect against multiple broad coronaviruses from Dr. Anthony Fauci and other key opinion leaders [12]. Morens et al argue that the development of pan-coronavirus vaccines necessitates the continued sampling of bat and other animal species and sequencing of coronaviruses to determining cross-reacting epitopes, as well as the enhanced study of human common cold coronaviruses to further characterize elicited immune responses and determine “cross-reactive and cross-protective epitopes” [12].

A pan-coronavirus vaccine design likely needs to be T-cell based for multiple reasons. First, based on our data and others in the literature, we know that T cell responses are robust, very broad, and have increased persistence for all known human coronaviruses (endemic and pandemic causing coronaviruses) and COVID-19 vaccinations. T cells also are able to cross-recognize VOCs including Omicron despite minimal neutralizing antibody cross-reactivity [13], suggesting that protection against severe disease in breakthroughs is partly a result of T cells. Second, we know from

animal models of coronaviruses that T cells are sufficient for viral clearance, and that animals were protected from fatal doses of infections without antibodies [14-16]. Third, from a logistical viewpoint, it is likely more challenging to develop neutralizing antibodies against diverse coronaviruses since most use different host receptors. Even within the closely related sarbecovirus subgenus that SARS-CoV-2 is classified in, there are differences in sequence of the receptor binding domain [3,17,18]. If alphacoronaviruses, which infect mammals and include HCoV-NL63 and HCoV-229E are included, the RBD is very divergent and will make antibody targeting more challenging. Moreover, current human coronaviruses use different host receptors (for example MERS-CoV and SARS-CoV-2, which are both in the betacoronavirus subgenus) [17], and even HCoV-NL63 that uses the same ACE2 receptor is divergent and binds ACE2 in a different conformational mechanism than SARS-CoV-2 [18]. Furthermore, any mutations that arise might abrogate neutralizing antibody responses as is seen with VOCs [19,20], whereas T cell responses are more durable [6,7,13].

There is now progress and encouraging data in the development of T cell-based vaccines against SARS-CoV-2. In November, phase 1 data from the first peptide-based T cell vaccine (CoVac-1) showed that the vaccine elicited robust CD4+ and CD8+ T cell responses after 1 dose that surpassed natural infections and current COVID-19 vaccine regimens and comparably targeted POCs [21]. The vaccine uses HLA-DR-restricted peptides of various structural proteins and ORF-8, and further data will be important to demonstrate if T cell-based will be as effective as mRNA vaccines [21]. SARS-CoV-2 and SARS-CoV conserved CD8+ targeted peptides are also being developed as T cell-

based vaccines and will be important in demonstrating the efficacy of this vaccine platform against more diverse coronaviruses [22].

Further work is needed to characterize more cross-reactive CD4+ and CD8+ T cell epitopes across human coronaviruses and diverse coronaviruses. Our work identifying the S815-827 CD4+ T cell epitope is important because it is conserved in very diverse coronaviruses that are found in different genus, bind different host receptors, and even infect different hosts. Further work is needed to demonstrate protection elicited by this conserved epitope in vivo, likely using mouse models that have been previously used to demonstrate T cell efficacy [16,23].

10.3 References

1. Tregoning JS, Flight KE, Higham SL, Wang Z, Pierce BF. Progress of the COVID-19 vaccine effort: viruses, vaccines and variants versus efficacy, effectiveness and escape. *Nature Reviews Immunology.* 2021;21(10):626-636. https://doi.org/10.1038/s41577-021-00592-1. doi:10.1038/s41577-021-00592-1.

2. Lytras S, Xia W, Hughes J, Jiang X, Robertson DL. The animal origin of SARS-CoV-2. *Science.* 2021;373(6558):968-970. https://www.science.org/doi/abs/10.1126/science.abh0117. doi:10.1126/science.abh0117.

3. Letko M, Seifert SN, Olival KJ, Plowright RK, Munster VJ. Bat-borne virus diversity, spillover and emergence. *Nature Reviews Microbiology.* 2020;18(8):461-471. https://doi.org/10.1038/s41579-020-0394-z. doi:10.1038/s41579-020-0394-z.

4. Woldemeskel BA, Kwaa AK, Garliss CC, Laeyendecker O, Ray SC, Blankson JN. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. *J Clin Invest.* 2020;130(12):6631-6638. doi:143120 [pii].

5. Dykema AG, Zhang B, Woldemeskel BA, et al. Functional characterization of CD4+ T cell receptors crossreactive for SARS-CoV-2 and endemic coronaviruses. *J Clin Invest.* 2021;131(10):e146922. doi: 10.1172/JCI146922. doi:146922 [pii].

6. Woldemeskel BA, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. *J Clin Invest.* 2021;131(10):e149335. doi: 10.1172/JCI149335. doi:149335 [pii].

7. Woldemeskel BA, Garliss CC, Blankson JN. mRNA Vaccine-Elicited Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)–Specific T Cells Persist at 6 Months and Recognize the Delta Variant. *Clin Infect Dis.* 2021:ciab915. https://doi.org/10.1093/cid/ciab915. Accessed 2/17/2022. doi:10.1093/cid/ciab915.

8. Woldemeskel BA, Dykema AG, Garliss C, Cherfils S, Smith KN, Blankson JN. CD4+ T-cells from COVID-19 mRNA vaccine recipients recognize a conserved epitope present in diverse coronaviruses. *J Clin Invest.* 2022. https://doi.org/10.1172/JCI156083. doi:10.1172/JCI156083.

9. Tseng HF, Ackerson BK, Luo Y, et al. Effectiveness of mRNA-1273 against SARS-CoV-2 Omicron and Delta variants. *Nat Med.* 2022. https://doi.org/10.1038/s41591-022-01753-y. doi:10.1038/s41591-022-01753-y.

10. Collie S, Champion J, Moultrie H, Bekker L, Gray G. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa. *N Engl J Med.* 2022;386(5):494-496. https://doi.org/10.1056/NEJMc2119270. doi:10.1056/NEJMc2119270.

11. Woldemeskel B, Garliss C, Aytenfisu T, et al. SARS-CoV-2 -specific immune responses in boosted vaccine recipients with breakthrough infections during the Omicron variant surge. (accepted for publication).

12. Morens DM, Taubenberger JK, Fauci AS. Universal Coronavirus Vaccines — An Urgent Need. *N Engl J Med.* 2021. https://doi.org/10.1056/NEJMp2118468. doi:10.1056/NEJMp2118468.

13. GeurtsvanKessel CH, Geers D, Schmitz KS, et al. Divergent SARS-CoV-2 Omicron–reactive T and B cell responses in COVID-19 vaccine recipients. *Sci Immunol.* 2022;7(69):eabo2202. https://doi.org/10.1126/sciimmunol.abo2202. doi:10.1126/sciimmunol.abo2202.

14. Jincun Z, Jingxian Z, Stanley P. T Cell Responses Are Required for Protection from Clinical Disease and for Virus Clearance in Severe Acute Respiratory Syndrome Coronavirus-Infected Mice. *J Virol.* 2010;84(18):9318-9325. https://doi.org/10.1128/JVI.01049-10. doi:10.1128/JVI.01049-10.

15. Rudragouda C, Craig F, Jincun Z, Meyerholz David K, Stanley P, Sandri-Goldin RM. Virus-Specific Memory CD8 T Cells Provide Substantial Protection from Lethal Severe Acute Respiratory Syndrome Coronavirus Infection. *J Virol.* 2014;88(19):11034-11044. https://doi.org/10.1128/JVI.01505-14. doi:10.1128/JVI.01505-14.

16. Zhao J, Zhao J, Mangalam AK, et al. Airway Memory CD4+ T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity.* 2016;44(6):1379-1391. https://www.sciencedirect.com/science/article/pii/S1074761316301601. doi:https://doi.org/10.1016/j.immuni.2016.05.006.

17. Verma J, Subbarao N. A comparative study of human betacoronavirus spike proteins: structure, function and therapeutics. *Arch Virol.* 2021;166(3):697-714. doi:10.1007/s00705-021-04961-y [doi].

18. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. *J Biol Chem.* 2020;295(37):12910-12934. doi:S0021-9258(17)49954-6 [pii].

19. Garcia-Beltran W, Lam EC, St Denis K, et al. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. *Cell.* 2021;184(9):2372-2383.e9. https://pubmed.ncbi.nlm.nih.gov/33743213 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7953441/. doi:10.1016/j.cell.2021.03.013.

20. World Health Organization. Tracking SARS-CoV-2 variants. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.

21. Heitmann JS, Bilich T, Tandler C, et al. A COVID-19 peptide vaccine for the induction of SARS-CoV-2 T cell immunity. *Nature.* 2022;601(7894):617-622. https://doi.org/10.1038/s41586-021-04232-5. doi:10.1038/s41586-021-04232-5.

22. Dolgin E. T-cell vaccines could top up immunity to COVID, as variants loom large. https://www.nature.com/articles/d41587-021-00025-3. Updated 2022.

23. Zhuang Z, Lai X, Sun J, et al. Mapping and role of T cell response in SARS-CoV-2–infected mice. *J Exp Med.* 2021;218(4):e20202187. https://doi.org/10.1084/jem.20202187. Accessed 3/18/2022. doi:10.1084/jem.20202187.

11 Curriculum Vitae

Bezawit A. Woldemeskel
bwoldem1@jhmi.edu | www.linkedin.com/in/beza-woldemeskel

EDUCATION

Johns Hopkins University School of Medicine, Baltimore, MD 2017 - 2022
PhD Candidate in the Cellular and Molecular Medicine Program
Laboratory of Joel Blankson MD, PhD
Department of Medicine, Division of Infectious Diseases

Mount Holyoke College, South Hadley, MA
2010 - 2014
Bachelor of Arts in Biochemistry
Cum Laude with High Honors in Biochemistry
GPA: 3.64/4.0

RESEARCH EXPERIENCES

Doctoral Research:

Johns Hopkins University School of Medicine Sept 2017 – 2022
Department of Infectious Disease. Mentor: Joel Blankson, MD, PhD, Professor of Medicine

- Characterized T cell responses to endemic common cold coronaviruses and SARS-CoV-2 in healthy donors, COVID-19 convalescent patients (CCPs) and people living with HIV
- Identified and characterized cross-reactive T cell responses that can cross-recognize endemic coronaviruses and SARS-CoV-2

Pre-doctoral Research Experience:

Research Associate, Selecta Biosciences Inc., Watertown, MA July 2014 – Apr 2017

- Developed bioassays to determine drug targeting profiles for vaccine development, gene therapy, and immune suppression projects, which are now in phase 1 and 2 clinical trials
- Conducted up to 50 enzyme-linked immunosorbent assays (ELISAs) daily as a part of a high throughput team
- Plan and execute plate-based assays to analyze cytokine content in spleens from animal experiments
- Process, analyze and present data generated from assays using different software such as GraphPad Prism and Softmax Pro
- Support in animal handling procedures such as: restraining for subcutaneous injections, cardiac puncture and tail vein bleed for blood collection, ear tagging for identification, spleen and lymph node isolation for *ex vivo* studies
- Trained junior scientists, and assisted in recruiting and interviewing candidates for the team

Selected Undergraduate Research Experience:

Honors Thesis, Woodard Lab, Mount Holyoke College Sep 2013 – May 2014

- Planned, executed, and analyzed experiments to study signaling mechanisms during *Drosophila melanogaster* development
- Presented, wrote, and defended a senior thesis project titled "*Coordination and cross talk between insulin signaling and ecdysone signaling during Drosophila melanogaster development"*

CURE Program Intern, Dana Farber / Harvard Cancer Center June 2013 – Aug 2013

- Utilized molecular biology techniques to study the regulation of DNA damage repair in immune cells
- Presented findings at research symposiums hosted by Harvard Medical School and Mount Holyoke College

Immunology Intern, Selecta Biosciences Inc., Watertown, MA Summer / Winter 2012

- Conducted bioassays in support of nicotine, malaria and immune tolerance vaccine development projects

PUBLICATIONS

1. **Woldemeskel BA**, Garliss CC, Aytenfisu TY, et al. SARS-CoV-2 -specific immune responses in boosted vaccine recipients with breakthrough infections during the Omicron variant surge. *JCI Insight.* 2022.
2. **Woldemeskel BA**, Dykema AG, Garliss CC, Cherfils S, Smith KN, Blankson JN. CD4+ T cells from COVID-19 mRNA vaccine recipients recognize a conserved epitope present in diverse coronaviruses. *J Clin Invest.* 2022;132(5):e156083.
3. **Woldemeskel BA***, Garliss CC*, Aytenfisu TY, et al. Discordant antibody and T cell responses to the SARS-CoV-2 Omicron variant in COVID-19 mRNA vaccine recipient. *Clin Infect Dis.* 2022.
4. Park HS, Shapiro JR, Sitaras I**, Woldemeskel BA** et al. Adaptive immune responses in vaccinated patients with symptomatic SARS-CoV-2 Alpha infection. *JCI Insight.* 2022;7(5):e155944. doi: 10.1172/jci.insight.155944.
5. **Woldemeskel BA*,** Garliss CC*, Blankson JN. mRNA Vaccine-Elicited SARS-CoV-2-Specific T cells Persist at 6 Months and Recognize the Delta Variant. *Clin Infect Dis.* 2021.
6. **Woldemeskel BA,** Kwaa AK, Blankson JN. Viral reservoirs in elite controllers of HIV-1 infection: Implications for HIV cure strategies. *EBioMedicine.* 2020;62:103118.
7. **Woldemeskel BA**, Karaba AH, Garliss CC, et al. The BNT162b2 mRNA Vaccine Elicits Robust Humoral and Cellular Immune Responses in People Living With Human Immunodeficiency Virus (HIV). *Clin Infect Dis.* 2022;74(7):1268-1270.
8. Dykema AG, Zhang B, **Woldemeskel BA**, et al. Functional characterization of CD4+ T cell receptors crossreactive for SARS-CoV-2 and endemic coronaviruses. *J Clin Invest.* 2021;131(10):e146922.

9. **Woldemeskel BA*,** Kwaa AK*, Garliss CC, Laeyendecker O, Ray SC, Blankson JN. Healthy donor T cell responses to common cold coronaviruses and SARS-CoV-2. *J Clin Invest.* 2020;130(12):6631-6638.
10. **Woldemeskel BA**, Garliss CC, Blankson JN. SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCoV-NL63. *J Clin Invest.* 2021;131(10):e149335.
11. Dykema AG, Zhang B, **Woldemeskel BA**, et al. SARS-CoV-2 vaccination diversifies the CD4+ spike-reactive T cell repertoire in patients with prior SARS-CoV-2 infection. *EBioMedicine.* 2022;80:104048.

CONFERENCES AND MEETINGS

Conference on Retroviruses and Opportunistic Infections (CROI)	February 2022
Conference on Retroviruses and Opportunistic Infections (CROI)	March 2021
Department of Immunology, Floor Meeting	November 2021
Department of Immunology, Floor Meeting	October 2020
Partnering Toward Discovery Seminar, Johns Hopkins Medicine	May 2020
Cellular and Molecular Medicine Program (CMM) Annual Retreat	September 2020
Department of Cell Biology, Lewis Talks	June 2019
Senior Symposium, Mount Holyoke College	April 2014
Leap Symposium, Mount Holyoke College	October 2013
CURE program summer presentation, Harvard Medical School	Aug 2013

AWARDS and HONORS

The Martin & Carol Macht Research Award, Young Investigators Day — March 2022
- Awarded for outstanding research by trainees from Johns Hopkins School of Medicine

New Investigator Scholarship, CROI Conference — March 2021

New Investigator Scholarship, CROI Conference — February 2022

High Honors in Biochemistry, Mount Holyoke College — May 2014
- Awarded for outstanding senior thesis project from Mount Holyoke College

Mary Lyon Scholar, Mount Holyoke College — May 2014
- Awarded for excellent academic performance and honors research

STUDENT LEADERSHIP EXPERIENCES

President, Biomedical Scholars Association, Johns Hopkins University — 2019 - 2020

- Led the largest student organization that supports underrepresented minority (URM) students at Johns Hopkins School of Medicine, Nursing and Public health with a 7-person executive board
- Planned and executed over 35 social, community service and career development events over two years that were attended by 50+ students, and featured deans and invited keynote speakers
- Increased attendance of events by implementing advertising strategies that included a monthly newsletter
- Organized 20+ coffee hour events for PhD recruitment for all SOM PhD programs
- Applied for and secured a $50,000 grant for the development of a mentoring network that supports URM students in collaboration with faculty and career offices
- Planned 20 recruitment events targeted towards increasing matriculation of URM students for 10 graduate programs
- Co-organized a mentoring program for 22 undergraduate students attending the Summer Internship Program (SIP) at Hopkins including recruiting graduate mentors
- BSA was awarded the 2021 Diversity Recognition Award for work during my tenure (2019-2021)

President-Elect, Biomedical Scholars Association (BSA) 2018 – 201
- Served on an executive team of 5 to plan and execute events aimed at supporting students from underrepresented backgrounds in School of Medicine, School of Nursing and School of Public Health

Committee member, Second Look Planning Committee 2019 – 2022
- Served on a committee with Damani Piggott MD, PhD to plan and execute an event for recruiting PhD students from underrepresented backgrounds to School of Medicine PhD programs

TEACHING AND MENTORING EXPERIENCES

Mentor, Blankson Lab, Johns Hopkins Medicine Summer 2020 & 2021
- Supervised two undergraduate students participating in the Hopkins Summer Internship Program (SIP)
- Assisted their preparation of poster and oral presentations which were presented at national conferences
- One student presented work at a national conference (ABRCMS) and won a poster presentation award

Mentor, Enroot, Cambridge, MA Aug 2015 – May 2017
- Mentored two high school students and recent immigrants through weekly tutoring sessions
- Assisted in applications to colleges, scholarships, and summer internships

Tutor, Project Literacy -Watertown Free Public Library, Watertown, MA Aug 2015 – May 2017
- Designed and planned weekly English lessons to an adult immigrant from China focused on improving pronunciation

Teaching Assistant, Biochemistry Department at Mount Holyoke College Sep 2013 - Dec 2013

- Assisted an advanced biochemistry class in the safe execution of weekly experiments

COMMUNITY SERVICE AND VOLUNTEER EXPERIENCES

Emergency Department Volunteer, Cooley Dickenson Hospital Sep 2013 - Jan 2014

- Served as a liaison between emergency room patients and medical staff by answering patient call bells and communicating immediate concerns
- Provided comfort measures such as food, water, blankets, and companionship to patients
- Maintained emergency rooms by stocking supplies and disinfecting rooms

Volunteer, Massachusetts Eye and Ear Infirmary, MA Jun 2012 – Aug 2012

- Assisted administrative staff by compiling necessary paperwork for patients and answering phone calls
- Ensured patient comfort and transported patients to different floors

UNDERGRADUATE WORK EXPERIENCES

***Fitness Center Monitor*,** Kendall Sports Complex, Mount Holyoke Sep 2011 – Dec 2013

- Managed the help desk and assisted fitness center customers in the safe use of exercise equipment
- Enforced fitness center rules and regulations and attended to questions and queries presented by customers

Student Advisor, Residential Life at Mount Holyoke College Aug 2011 – May 2012

- Implemented housing policies, and managed the general welfare of a floor of 38 students
- Created an inclusive community for residents by planning and organizing floor activities, and making informative bulletin boards
- Mentored first year students on academic policies and mediated roommate conflicts

Hall Senator, Student Government Association at Mount Holyoke College Sep 2011 – Dec 2011

- Served as a liaison between students and the SGA by representing Rockefeller Hall residents in Student Government Association