TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

COVID-19 VACCINE-INDUCED ADAPTIVE IMMUNITY AGAINST SARS-CoV-2 VARIANTS

Pinja Jalkanen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1746 | MEDICA – ODONTOLOGICA | TURKU 2023

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

COVID-19 VACCINE-INDUCED ADAPTIVE IMMUNITY AGAINST SARS-CoV-2 VARIANTS

Pinja Jalkanen

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
SARJA – SER. D OSA – TOM. 1746 | MEDICA – ODONTOLOGICA | TURKU 2023

University of Turku

Faculty of Medicine
Institute of Biomedicine
Virology
Turku Doctoral Programme of Molecular Medicine (TuDMM)

Supervised by

Professor Ilkka Julkunen, MD, PhD
Institute of Biomedicine / Virology
University of Turku
Turku, Finland

Docent Laura Kakkola, PhD
Institute of Biomedicine / Virology
University of Turku
Turku, Finland

Reviewed by

Docent Tuija Leino, MD, PhD
Department of Health Security
Finnish Institute for Health and Welfare
Helsinki, Finland

Docent Tomas Strandin, PhD
Department of Virology
University of Helsinki
Helsinki, Finland

Opponent

Docent Maria Söderlund-Venermo, PhD
Department of Virology
University of Helsinki
Helsinki, Finland

ISBN 978-951-29-9464-9 (PRINT)
ISBN 978-951-29-9465-6 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama, Turku, Finland 2023

To my family

UNIVERSITY OF TURKU
Faculty of Medicine
Institute of Biomedicine
Virology
PINJA JALKANEN: COVID-19 vaccine-induced adaptive immunity against SARS-CoV-2 variants
Doctoral dissertation, 145 pp.
Turku Doctoral Programme of Molecular Medicine (TuDMM)
October 2023

ABSTRACT

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has necessitated the rapid development and deployment of vaccines worldwide. mRNA-based vaccines against COVID-19 have demonstrated a high degree of efficacy against the original SARS-CoV-2 strain. However, the emergence of several genetic variants of the virus presents significant challenges to worldwide efforts in controlling the pandemic. To date, five dominant variants, namely Alpha, Beta, Gamma, Delta, and Omicron, have caused major epidemic waves of SARS-CoV-2 infections. Understanding the adaptive immune responses induced by COVID-19 vaccines against these variants is crucial for evaluating vaccine effectiveness and informing public health strategies.

COVID-19 mRNA vaccines stimulate robust adaptive immune responses characterized by the production of antibodies targeting the spike (S) protein of SARS-CoV-2 and the generation of memory T cells. In this study, we developed an immunoassay based on SARS-CoV-2 proteins and a live virus microneutralization test (MNT) to measure the production and persistence of vaccine-induced and naturally acquired neutralizing antibodies. Additionally, we employed an activation-induced marker (AIM) assay to analyse the activation of memory T cells against SARS-CoV-2 variants. Immune responses were studied in Finnish healthcare workers.

The results of this study demonstrate that the completion of a two-dose mRNA vaccine regimen leads to consistent and high production of S-specific antibodies across all studied age groups. Vaccinated individuals exhibit the ability to neutralize the SARS-CoV-2 Alpha variant, while neutralization against the Beta and Delta variants is reduced. Furthermore, a majority of vaccinated participants display the presence of SARS-CoV-2 S-specific memory CD4+ and CD8+ T cells, which exhibit cross-recognition of the different SARS-CoV-2 variants. S-specific antibody levels were found to decline within months following vaccination, while T cell responses remained stable for at least six months.

These results highlight the durability of memory T cell responses and their role in providing sustained protection against SARS-CoV-2 variants.

KEYWORDS: SARS-CoV-2, COVID-19, vaccine response, adaptive immune response, genetic variant, pandemic

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Biolääketieteen laitos
Virusoppi
PINJA JALKANEN: COVID-19 rokotteiden aikaansaama immuniteetti SARS-CoV-2-virusvariantteja vastaan
Väitöskirja, 145 s.
Molekyylilääketieteen tohtoriohjelma
Lokakuu 2023

TIIVISTELMÄ

Hengitystieinfektioita aiheuttavan uuden SARS-CoV-2-koronaviruksen ilmaantuminen johti maailmanlaajuisen COVID-19-pandemiaan, jonka hillitseminen on vaatinut nopeaa rokotteiden kehittämistä ja käyttöönottoa. COVID-19 mRNA-rokotteet ovat osoittautuneet tehokkaiksi alkuperäistä SARS-CoV-2-virusta vastaan, mutta uusien geneettisten varianttien ilmaantuminen on asettanut haasteita pandemian hallinnalle. Tähän mennessä viisi dominoivaa varianttia – Alpha-, Beta-, Gamma-, Delta- ja Omikron-variantit - ovat aiheuttaneet merkittäviä SARS-CoV-2-virusinfektioiden aaltoja. COVID-19-rokotuksessa syntyvän hankitun immuniteetin ymmärtäminen on ratkaisevan tärkeää rokotteen tehokkuuden sekä tulevien rokotusstrategioiden ja uusintarokotustarpeen arvioinnissa.

COVID-19 mRNA-rokotteet saavat aikaan voimakkaan immuunivasteen, mukaan lukien SARS-CoV-2-viruksen piikkiproteiini-spesifisten vasta-aineiden tuotannon ja muisti-T-solujen kehittymisen. Tässä väitöskirjassa pystytettiin virusproteiineja tunnistava vasta-ainetesti ja elävää SARS-CoV-2-virusta hyödyntävä mikroneutralisaatiotesti mittaamaan rokotteen ja luonnollisen infektion synnyttämien vasta-aineiden määrää sekä niiden kykyä neutraloida SARS-CoV-2-variantteja. Lisäksi työssä hyödynnettiin T-solujen aktivaatiomääritystä, jolla analysoitiin muisti-T-solujen kykyä tunnistaa muuntuneita SARS-CoV-2-variantteja. Rokotevasteita tutkittiin suomalaisissa terveydenhuollon työntekijöissä.

Tämän väitöskirjan tulokset osoittivat, että kahden rokoteannoksen jälkeen kaikki tutkittavat tuottivat vasta-aineita S-proteiinia kohtaan iästä ja sukupuolesta riippumatta. Vasta-ainevaste oli yhtä voimakas Alpha-varianttia kohtaan kuin alkuperäistä SARS-CoV-2-virusta kohtaan, kun taas Beta- ja Delta-varianttien neutralisaatio oli heikentynyt sekä rokotetuilla että luonnollisen taudin sairastaneilla. Suurimmalla osalla rokotetuista henkilöistä voitiin todeta SARS-CoV-2-viruksen S-proteiinin tunnistavia auttaja- ja sytotoksisia-T-soluja. Lisäksi T-solut tunnistivat virusvariantteja yhtä hyvin kuin alkuperäistä virusta. Vasta-ainetasot laskivat muutamien kuukausien kuluessa rokotuksesta, kun taas muisti-T-soluaktivaation havaittiin säilyvän samalla tasolla vielä kuuden kuukauden kuluttua rokotuksesta.

Nämä tulokset osoittavat, että mRNA-rokotteet tarjoavat kestävän suojan SARS-CoV-2-virusvarianttien aiheuttamaa tautia vastaan muisti-T-solujen välityksellä.

AVAINSANAT: COVID-19, SARS-CoV-2-virus, rokotevaste, virusmuunnos

Table of Contents

Abbreviations

aa	amino acid
ACE2	angiotensin-converting enzyme 2
AIM	activation-induced marker
APC	antigen presenting cell
AUC	area under curve
BSL3	biosafety level 3
CDC	U.S. Centers for Disease Control and Prevention
COVID-19	coronavirus disease 2019
DMSO	dimethyl sulfoxide
E	envelope protein
EIA	enzyme immunoassay
EMA	European Medicines Agency
FBS	fetal bovine serum
FCS	furin cleavage site
FDA	U.S. Food and Drug Administration
FL	fusion loop
GC	germinal centre
GM	geometric mean
GST	glutathione S-transferase
hCoV	human coronaviruses
HCW	healthcare worker
HEK293F	human embryonal kidney cells
HR	heptapeptide repeat sequence
HUS	Helsinki University Hospital
ICTV	International Committee on Taxonomy of Viruses
ID50	inhibitory dose of 50%
IFN	interferon
Ig	immunoglobulin
IL	interleukin
M	membrane protein
MERS	Middle East respiratory syndrome coronavirus

mFc	mouse monoclonal IgG2a Fc part
MHC	major histocompatibility complex
MNT	microneutralization test
N	nucleoprotein
nsp	non-structural protein
NTD	N-terminal domain
OD	optical density
ORF	open reading frame
PBMC	peripheral blood mononuclear cell
pp	polyprotein
proMstn	mouse myostatin growth factor proregion
RBD	receptor binding domain
RdRp	RNA-dependent RNA polymerase
ROC	receiver operating characteristic
RTC	replicase-transcriptase complex
RT-PCR	reverse transcription polymerase chain reaction
S	spike protein
S1	spike protein subunit 1
S2	spike protein subunit 2
SARS	severe acute respiratory syndrome coronavirus
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
SD1	spike protein subdomain 1
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sf-9	*Spodoptera frugiperda* cells
SI	stimulation index
TCID50	fifty-percent tissue culture infective dose
Th	T helper cell
THL	Finnish Institute for Health and Welfare
TM	transmembrane domain
TMPRSS2	transmembrane serine protease 2
TNFα	tumor necrosis factor alpha
TYKS	Turku University Hospital
VE	vaccine efficiency
VeroE6	African green monkey kidney cells clone E6
WHO	World Health Organization
VLP	virus-like particle
VOC	variant of concern
VOI	variant of interest
VUM	variant under monitoring

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Jalkanen, P., Pasternack, A., Maljanen, S., Melén, K., Kolehmainen, P., Huttunen, M., Lundberg, R., Tripathi, L., Khan, H., Ritvos, M. A., Naves, R., Haveri, A., Österlund, P., Kuivanen, S., Jääskeläinen, A. J., Kurkela, S., Lappalainen, M., Rantasärkkä, K., Vuorinen, T., Hytönen, J., Waris, M., Tauriainen, S., Ritvos, O., Kakkola, L., & Julkunen, I. A Combination of N and S Antigens with IgA and IgG Measurement Strengthens the Accuracy of SARS-CoV-2 Serodiagnostics. *The Journal of Infectious Diseases*, 2021.

II Jalkanen, P., Kolehmainen, P., Häkkinen, H. K., Huttunen, M., Tähtinen, P. A., Lundberg, R., Maljanen, S., Reinholm, A., Tauriainen, S., Pakkanen, S. H., Levonen, I., Nousiainen, A., Miller, T., Välimaa, H., Ivaska, L., Pasternack, A., Naves, R., Ritvos, O., Österlund, P., Kuivanen, S., Smura, T., Hepojoki, J., Vapalahti, O., Lempainen, J., Kakkola, L., & Julkunen, I. COVID-19 mRNA vaccine induced antibody responses against three SARS-CoV-2 variants. Nature Communications, 2021.

III Jalkanen, P., Kolehmainen, P., Haveri, A., Huttunen, M., Laine, L., Österlund, P., Tähtinen, P. A., Ivaska, L., Maljanen, S., Reinholm, A., Belik, M., Smura, T., Häkkinen, H. K., Ortamo, E., Kantele, A., Julkunen, I., Lempainen, J., & Kakkola, L. Vaccine-Induced Antibody Responses against SARS-CoV-2 Variants-Of-Concern Six Months after the BNT162b2 COVID-19 mRNA Vaccination. Microbiology Spectrum, 2022.

IV Hurme, A.*, Jalkanen, P.*, Heroum, J., Liedes, O., Vara, S., Melin, M., Teräsjärvi, J., He, Q., Pöysti, S., Hänninen, A., Oksi, J., Vuorinen, T., Kantele, A., Tähtinen, P. A., Ivaska, L., Kakkola, L., Lempainen, J., & Julkunen, I. Long-Lasting T Cell Responses in BNT162b2 COVID-19 mRNA Vaccinees and COVID-19 Convalescent Patients. *Frontiers in Immunology,* 2022. *equal contribution

1 Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing infectious coronavirus disease 2019 (COVID-19) emerged at the end of 2019, likely through zoonotic transmission (Pekar et al., 2022; Worobey et al., 2022). The subsequent SARS-CoV-2 outbreak was declared a pandemic by the World Health Organization (WHO), and as of August 2023, the ongoing pandemic had resulted in more than 770 million infections and 6.9 million deaths (WHO, 2023b). SARS-CoV-2 is closely related to two previously identified highly pathogenic human coronaviruses (hCoVs) with a zoonotic origin, SARS and Middle East respiratory syndrome (MERS) coronaviruses. In addition, repeated spillover events of animal coronaviruses to humans have led to the circulation of four low pathogenic hCoVs that cause the common cold: OC43, HKU1, 229E, and NL63.

SARS-CoV-2 has a large RNA genome that undergoes frequent recombination and mutation events, despite the proofreading mechanism (Markov et al., 2023). Starting in late 2020, several genetic variants with distinct characteristics have emerged, including increased infectivity and virulence, higher transmissibility, and the ability to evade adaptive immunity. The variants that pose the most significant risk to global public health have been classified as variants of concern (VOCs) by WHO. To date, five variants have been defined as VOCs: Alpha, Beta, Gamma, Delta, and Omicron.

Neutralizing antibodies primarily target the spike (S) protein (Greaney et al., 2021; L. Liu et al., 2020; Seydoux et al., 2020), making S the target of most COVID-19 vaccines that have been authorized or are in the development phase. Many COVID-19 vaccines have been shown to induce CD4+ helper and CD8+ cytotoxic T cell responses, which have been associated with protection against the severe forms of the disease (Painter et al., 2021; Zuo et al., 2021). However, T cell-mediated responses alone cannot prevent the infection, and the rapid decrease of neutralizing antibodies has necessitated the administration of booster vaccine doses. Furthermore, since SARS-CoV-2 constantly mutates to evade vaccine-induced neutralizing antibodies, it is crucial to constantly monitor emerging variants, memory cell responses, and vaccine effectiveness.

The primary objective of this study was to analyse humoral and cell-mediated immune responses induced by COVID-19 mRNA vaccines in healthy adults during the mass vaccination campaigns in Finland in 2021 and to evaluate the duration of these responses. A secondary objective was to investigate the immune evasion of emerging variants circulating during 2020–2021. Additionally, we aimed to characterize the cross-reactivity of pre-pandemic hCoV-specific antibodies with SARS-CoV-2 to gain insights into the potential pre-existing immunity against COVID-19.

2 Review of the Literature

2.1 SARS-CoV-2

SARS-CoV-2 emerged in December 2019 in Wuhan, China, and it belongs to the order *Nidovirales*, family *Coronaviridae* and subfamily *Orthocoronavirinae*. The coronaviridae study group of the International Committee on Taxonomy of Viruses (ICTV) recognizes four distinctive genera in *Orthocoronavirinae* subfamily, *Alpha, Beta, Gamma,* and *Deltacoronavirus* (Woo et al., 2023). Alpha and Betacoronaviruses infect mammals, while Gamma and Deltacoronaviruses infect mainly avian species and are not found in humans. SARS-CoV-2 belongs to the *Betacoronavirus* genera and shares the highest sequence identity with two other Betacoronaviruses infecting humans, SARS and MERS. SARS-CoV-2 is the third coronavirus outbreak in the twenty-first century since SARS caused a severe pulmonary disease epidemic in 2003 with a 10% mortality rate (Chan-Yeung & Xu, 2003; Donnelly et al., 2003) and MERS in 2012 with a 36% mortality rate (WHO, 2019).

Four other hCoVs, Betacoronaviruses OC43 and HKU1 and Alphacoronaviruses 229E and NL63, typically cause mild upper respiratory tract infections and seasonal epidemics. 229E and OC43 were first isolated in the 1960s (Hamre & Procknow, 1966; McIntosh et al., 1967), while NL63 and HKU1 were discovered later, in 2004 (van der Hoek et al., 2004) and 2005 (Woo et al., 2005), respectively. The major natural reservoir host of coronaviruses is bats, where SARS, MERS, SARS-CoV-2, 229E, and NL63 are thought to originate before transmission to other animals that function as intermediate hosts (Cui et al., 2019). The intermediate host of NL63 is not known; however, 229E is likely transmitted to humans from alpacas (Crossley et al., 2012), SARS from palm civets (Guan et al., 2003; Kan et al., 2005), and MERS from dromedary camels (Mohd et al., 2016). In contrast, OC43 and HKU1 are thought to originate in rodents, and the intermediate host of OC43 is likely cows (Bidokhti et al., 2013).

Previous studies have shown that SARS-related bat viruses have the ability to infect human cells (Temmam et al., 2022). SARS-CoV-2 shares over 95% whole genome sequence identity with the bat coronavirus RaTG13 (P. Zhou et al., 2020), while receptor binding domain (RBD) has the highest identity with coronavirus

isolated from pangolin in 2019 (Boni et al., 2020). However, there are no signs of direct recombination with the pangolin coronavirus, indicating multiple recombination events in SARS-CoV-2 evolution (Hassanin et al., 2022). Despite extensive research, the indeterminate host animal and the timing when the virus crossed the species barrier have not been determined.

2.1.1 Genomic structure

SARS-CoV-2 is a large, enveloped virus with a positive-sense single-stranded RNA genome. The genome is non-segmented, around 30,000 bp long, and multiple open reading frames (ORFs) encode four structural proteins and 25 non-structural or accessory proteins (C. Wu et al., 2022) (Figure 1). Two-thirds of the 5' end of the genome is a replicase gene comprising two overlapping ORFs (ORF1a and ORF1b), which encode polyproteins (pp1a and pp1ab). Pp1a and pp1ab are further processed by two virus-encoded proteinases into 16 non-structural proteins (nsp1-nsp16) (V'kovski et al., 2021). Nsps have independent functions, including host innate immune evasion, proteolytic cleavage of pp1a and pp1ab, and RNA proofreading. Nsp2-16 compose the replicase-transcriptase complex (RTC) involved in synthesizing full-length genomic RNA and subgenomic RNAs, which are used to transcribe structural and accessory proteins. ORF1a, ORF1b, and structural proteins are conserved in the Orthocoronavirus genome (Woo et al., 2023).

Structural proteins include the envelope protein (E), membrane protein (M), and spike glycoprotein (S), which forms the virion surface, and nucleoprotein (N), which packs around the viral RNA comprising the nucleocapsid structure. E-protein is the smallest of structural proteins and functions as an ion channel in addition to participating in virus maturation and budding (S. Zhou et al., 2023). M-protein is the most abundant structural protein and the main factor in virus assembly (Z. Zhang et al., 2022). N-protein is critical for packaging viral genome, protecting it from the host cell environment, and regulating gene transcription (C.-H. Wu et al., 2014). However, most of the research efforts have been targeted at S-protein responsible for receptor recognition, attachment, and entry into the host cell (Huang et al., 2020). After binding to the host cell receptor angiotensin-converting enzyme 2 (ACE2), transmembrane protease serine 2 (TMPRSS2) located on the host cell membrane promotes the S-protein activation by cleaving it into spike subunit 1 (S1) and membrane-fusion subunit 2 (S2). S1 includes N-terminal domain (NTD), receptor binding domain (RBD), and subdomains 1 and 2 (SD1 and SD2), while S2 includes fusion loop (FL), heptapeptide repeat sequence 1 and 2 (HR1 and HR2), transmembrane domain (TM), and intracellular tail. S2 is more conserved between Orthocoronaviruses compared to S1 (Woo et al., 2023).

Nine accessory proteins, ORF3a, ORF3b, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10, are encoded from overlapping ORFs with structural genes. Accessory proteins are not necessary for virus replication; however, some are involved in viral pathogenesis, while the functions of some ORFs have so far remained uncharacterized (Redondo et al., 2021). Recent studies have shown that ORF3b, ORF6, ORF7a, ORF7b, ORF8, and ORF9b are type I interferon (IFN) antagonists with diverse mechanisms (Min et al., 2021; Redondo et al., 2021).

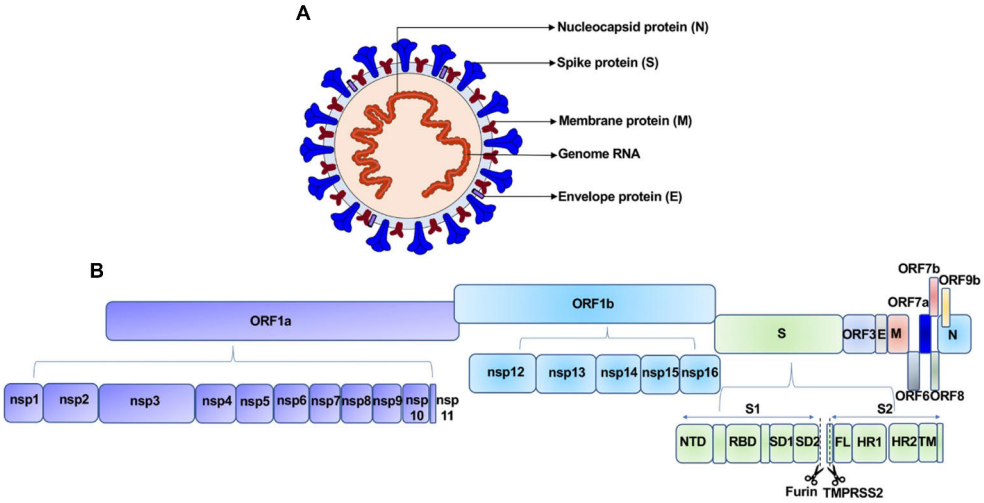

Figure 1. SARS-CoV-2 virus particle and genomic structure. **A.** Four structural proteins and positive-sense single-stranded RNA genome. **B.** Open reading frames 1a (ORF1a) and 1b (ORF1b) encode sixteen nonstructural proteins (nsp1–11 and nsp12–16). S encodes N-terminal domain (NTD), receptor-binding domain (RBD), subdomain 1 (SD1) and 2 (SD2), fusion loop (FL), heptapeptide repeat sequence 1 (HR1) and 2 (HR2), and transmembrane domain (TM). S is cleaved into subunits 1 (S1) and 2 (S2) by furin and transmembrane serine protease 2 (TMPRSS2). Modified from Zhang et al. 2021.

2.1.2 Modes of evolution

The first SARS-CoV-2 whole genome sequence, designated Wuhan-Hu-1, was made publicly available already at the beginning of January 2020 (F. Wu et al., 2020). Since then, constant surveillance has resulted in an unprecedented number of 16 million sequences submitted into the GISAID EpiCoV repository (https://gisaid.org/hcov19-variants/). In Finland, SARS-CoV-2 variants have been monitored with whole genome sequencing since December 2020 (Finnish Institute for Health and Welfare, 2021). As with most RNA viruses, SARS-CoV-2 evolves relatively rapidly, and in three years, the virus has diverged into multiple competing genetic variants, with a new emerging variant displacing the previous globally dominant strain every two to six months (https://gisaid.org/hcov19-variants/).

SARS-CoV-2 evolution is driven by mutation rates, random genetic changes, natural selection, and recombination. In SARS-like coronaviruses, nucleotide substitutions occur at similar rates, and studies have estimated the mutation rates to be 0.8–4.7x10^{-3} nucleotide substitutions per site per year (Chaw et al., 2020; J.-F. He et al., 2004; Z. Zhao et al., 2004) or 1.3x10^{-6} nucleotide substitutions per replication cycle (Amicone et al., 2022). These mutation rates are lower than in many other RNA viruses, such as influenza and hepatitis C, which lack exonuclease proofreading mechanisms (Pauly et al., 2017; Ribeiro et al., 2012). In addition to nucleotide substitutions, which may result in changes in the amino acids (aa), RNA replication errors may lead to aa insertions and deletions. Most aa changes are deleterious, leading to failure in viral replication and removal by natural selection. However, those changes that are beneficial to the virus, i.e., they increase receptor binding, replication, transmission or evasion of host immune responses, are positively selected. Recombination is common for coronaviruses and requires co-circulation of the viruses and co-infection of the same host. In particular, the recombination of the S has significant effects on the virus fitness and the most recent dominant variant, Omicron XBB.1.5, is a recombinant between two Omicron sublineage viruses, BA.2.10.1 and BA.2.75.

SARS-CoV-2 evolution is also affected by epidemiological dynamics, including the size of the infected population, host interactions, types of transmission cycles, and population-level immunity. With SARS-CoV-2, the size of the infected population is massive, increasing the possibility of spontaneous mutations and the chances of these mutations being transmitted further. High vaccine coverage and the use of antiviral therapeutics add selective pressure to the virus. In addition, some studies suggest that chronic infections in immunocompromised patients treated with plasma or monoclonal antibody therapy allow the virus to evolve faster and promote mutations which increase the viral replication and immune evasions (Harari et al., 2022). SARS-CoV-2 has an ability to replicate in other species such as mice (Stone et al., 2022), domestic cats (Barrs et al., 2020), farmed minks (Oude Munnink et al., 2021), and white-tailed deer (Kuchipudi et al., 2022), and it is possible that after diversification in an animal host, some sublineages spillover back to humans carrying non-typical aa changes.

2.1.3 Variants of concern

SARS-CoV-2 variants are named based on at least four different nomenclature systems: GISAID and Nextstrain clades, Pango lineage, and WHO classification. Pango lineage is widely used and covers the entire diversity of SARS-CoV-2 (O'Toole et al., 2022), while WHO provides labels only for lineages most relevant to public health (https://www.who.int/activities/tracking-SARS-CoV-2-variants).

WHO classifies SARS-CoV-2 variants as variants under monitoring (VUM), variants of interest (VOI), and variants of concern (VOC) based on their potential to spread and replace previous variants. VUMs have genetic changes that are suspected to affect the virus fitness, and VOIs have changes that are predicted or known to affect virus transmissibility, disease severity, or immune escape, and their prevalence is increasing. In early 2021, for example, Epsilon (Pango lineage B.1.427 and B.1.429), Eta (B.1.525), and Iota (B.1.526) were designed as VOIs. VOCs are VOIs that pose the most significant risk to global public health and show a transmission advantage over the other circulating VOCs. By April 2023, five lineages were classified as VOCs: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.529).

The first divergent SARS-CoV-2 lineage, Alpha variant, appeared in September 2020 superseding the present lineage, which had only one substitution in the S protein, D614G. Beta and Gamma variants were recognized soon after in different parts of the world (Faria et al., 2021; Tegally et al., 2021), and all three cocirculated during spring 2021, with either Alpha, Beta, or Gamma being the most dominant ones in different geographic areas (C. Cohen et al., 2022; Naveca et al., 2021). Alpha was the most dominant in Finland and co-circulated with Beta until the summer of 2021 (Figure 2). Alpha, Beta, and Gamma had strikingly 14, 8, and 11 aa changes in the S protein, respectively, which contributed to 40–160% higher transmissibility than the original virus (Coutinho et al., 2021; Davies, Abbott, et al., 2021; S. Zhao et al., 2022). Delta variant emerged in late 2020 and became globally dominant during the summer of 2021 with critical mutations in the S protein (Earnest et al., 2022). Delta was 60% more transmissible than Alpha variant, and a study in Scotland determined that Delta infection led to hospital admission twice as likely as Alpha infection (Sheikh et al., 2021). Alpha, Beta, and Gamma were also associated with more severe disease forms than those with pre-existing variants (Lin et al., 2021). Furthermore, Alpha variant was associated with a 66% higher risk of death within 28 days of a positive PCR test compared to the original strain (Davies, Jarvis, et al., 2021).

The Omicron variant emerged in November 2021 with a high number of spike mutations, changing the course of the pandemic by leading to a massive global wave of infections (WHO, 2023b). In Finland, the number of SARS-CoV-2 infections increased rapidly and by the end of 2022, 54% of the Finnish adult population had been infected (Solastie et al., 2023). Before the emergence of Omicron variant, seroprevalence was estimated to be <7%. Omicron variant is the most diverse of all the VOCs, and its closest ancestor is B.1.1 lineage dating back to 2020 (Martin et al., 2022). The evolution of Omicron is unclear; whether it had circulated in an unmonitored population or emerged from an animal host or a chronic COVID-19 patient is unknown. A recent study determined that Omicron is ten times more transmissible than the original variant and it has a high capacity for immune escape

(Jiahui Chen et al., 2022). Omicron infects the upper respiratory tract in hamsters (Armando et al., 2022), indicating that Omicron sublineage viruses cause milder disease than pre-Omicron VOCs. A data linkage study in South Africa also detected a lower hospitalization risk in Omicron infected than non-Omicron infected patients (Wolter et al., 2022).

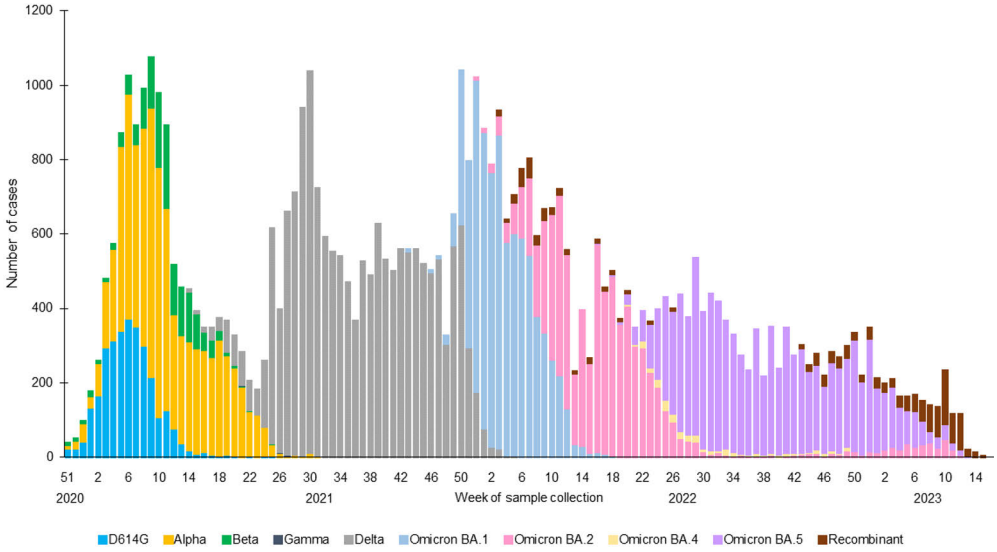

Figure 2. The weekly number of SARS-CoV-2 variants of concern (VOCs) sequenced in Finland from December 2020 to April 2023. Data are from the Finnish National Infectious Diseases Register maintained by the Finnish Institute for Health and Welfare (THL). Virus strains that were not VOCs are not shown.

2.2 COVID-19

2.2.1 Clinical manifestation

SARS-CoV-2 infection can be asymptomatic or symptomatic. Two meta-analyses estimated that approximately 16–40% of infected had no symptoms at the time of the diagnosis (J. He et al., 2021; Q. Ma et al., 2021). However, the actual number is unclear since some individuals developed symptoms later. Most symptomatic infections are mild, meaning that they do not require hospitalization. In September 2021, United States Centers for Disease Control and Prevention (CDC) estimated that 85% of the infections are mild, 5% require hospitalization, and 0.6% of the patients died (CDC, 2021). The risk of severe illness increases with age and the number of comorbidities, and varies by immunity status and circulating variants (Russell et al., 2023). Although Omicron-infected patients have a lower risk of

severe disease (Leiner et al., 2022) and vaccinations and antiviral treatments were available during the Omicron period, the COVID-19-associated hospitalizations increased in spring 2022 due to a massive number of infections (Havers et al., 2022). The risk of death is lower in those with at least two vaccine doses compared to those without vaccination or with only one vaccine dose (Tenforde et al., 2022).

The most common clinical symptoms caused by SARS-CoV-2 are fever, headache, loss of smell, nasal obstruction, cough, fatigue, muscle aches, runny nose, loss of appetite, and sore throat (Alimohamadi et al., 2020; Lechien et al., 2020). SARS-CoV-2 enters the body through the mucosal surfaces of the respiratory tract, and it is mainly spread through respiratory droplets and aerosols released during coughing, sneezing, or speaking. The incubation period of COVID-19 is typically four to five days after exposure; however, Omicron variant decreased incubation time to three days (Ogata & Tanaka, 2023; Y. Wu et al., 2022). The most common manifestation of severe disease is pneumonia, which can be complicated by respiratory failure requiring mechanical ventilation. A high risk of fatal COVID-19 has been associated with high viral load, release of pro-inflammatory cytokines, such as interleukin 6 (IL-6), IL-8 and tumor necrosis factor (TNF), and delayed or ineffective type I and III IFN responses (Galani et al., 2021). Inadequate interferon response seems to correlate with slowed or extreme activation of cellular immunity leading to more severe disease (Moss, 2022).

Although most individuals with COVID-19 recover from the illness within a few weeks, a subset of patients experience persistent health problems for at least three months following the initial infection, referred to as long COVID or post-acute COVID-19 syndrome. Some long COVID patients recover within months; however, the symptoms, such as fatigue, chest pain, loss of taste or smell, breathing difficulties, cognitive impairment, and nausea, can persist for years (Davis et al., 2021; Demko et al., 2022). While the incidence of long COVID varies within studies, the long-term symptoms appear more likely in hospitalized (59%) than in non-hospitalized (13–38%) (Augustin et al., 2021; Pérez-González et al., 2022) or vaccinated patients (10%) (Ayoubkhani et al., 2022). The underlying pathophysiological mechanisms of long COVID remain unclear. Several hypotheses have been proposed, including immune dysregulation, persistent SARS-CoV-2 infection in tissues, autoimmunity, endothelial dysfunction, and dysfunctional neurological signaling (Davis et al., 2023).

2.2.2 Testing for SARS-CoV-2 infection

Several diagnostic methods have been developed for the detection of SARS-CoV-2 infection. The most frequently used methods are nucleic acid amplification tests, such as reverse transcription polymerase chain reaction (RT-PCR), and antigen

tests (WHO, 2020). RT-PCR is used for the detection of SARS-CoV-2 RNA in upper respiratory tract specimens, such as nasal and nasopharyngeal swabs or saliva. PCR tests are sensitive and accurate, and they have become the golden standard in diagnosing SARS-CoV-2 as well as other respiratory virus infections (Puhach et al., 2023). Using at least two gene targets is important for accuracy, and common gene targets include ORF1ab, E, N, S, and RNA-dependent RNA polymerase (RdRp) (Vogels et al., 2020). Some variants, including Alpha and Omicron, have mutations in the S gene resulting in negative S gene target RT-PCR results, which can be used to distinguish Alpha or Omicron variants from other variants circulating at the same time (McMillen et al., 2022). RT-PCR allows the early detection of the virus, even in asymptomatic individuals, and the test can be positive already on the day of the infection (Kim et al., 2021; McEllistrem et al., 2021). However, PCR tests do not differentiate between infectious and non-viable viruses, and some patients have had positive RT-PCR test results several months after recovery (Henderson et al., 2021).

Rapid antigen tests detect the presence of viral proteins in respiratory tract specimens, indicating an active infection. Antigen tests can produce results in minutes and be used at home, near the patient, and in laboratory settings. Antigen tests are less sensitive than RT-PCR tests (Pickering et al., 2021) and they detect asymptomatic infections poorly (Mitchell et al., 2021). However, antigen tests are useful for screening in community settings due to their low cost, availability, and fast turnaround time.

A past SARS-CoV-2 infection or COVID-19 vaccination can be detected with serological assay, which detects the presence of SARS-CoV-2-specific antibodies in the blood. Most serological assays use N and S proteins or parts of S, mainly RBD or S1, which share less homology with seasonal hCoVs (Hicks et al., 2021). The technologies vary between non-quantitative rapid lateral flow tests and quantitative laboratory assays, where the binding of antibodies is detected based on enzymatic reaction, chemiluminescence, or fluorescence (WHO, 2020). Antibody tests should not be used for diagnosis; however, antibody measurement of saliva has been shown to increase the positive rates when combined with nucleic acid detection (S. Yu et al., 2022). Furthermore, testing of the acute phase and convalescent phase samples could aid with the diagnosis when there is continued clinical suspicion of COVID-19 despite negative nucleic acid amplification test results (WHO, 2020). Antibody tests are useful for surveillance studies and determining the prevalence of virus infections in the population. Serological tests have also been used in research to determine and compare the effectiveness of vaccines to induce antibody responses.

2.3 COVID-19 vaccinations

The development of COVID-19 vaccines started already in January 2020 due to the rapid spread of SARS-CoV-2. The first vaccines receiving conditional marketing authorization by European Medicines Agency (EMA) were Comirnaty (research name: BNT162b2, BioNTech and Pfizer) in December 2020 and Spikevax (mRNA-1273, Moderna) and Vaxzevria (ChAdOx1, AstraZeneca) in January 2021 (EMA, 2023). The primary vaccine series for all three vaccines included two intramuscularly administrated doses for adults. Currently, eight vaccines are approved in the EU, while only four are approved or under emergency use authorization by U.S. Food and Drug Administration (FDA, 2023a). By December 2022, 50 vaccines were approved at least in one country worldwide (McGill COVID19 Vaccine Tracker Team, 2022), and in 2023, WHO had granted 11 vaccines permission for emergency use (WHO, 2023a). COVID-19 vaccines utilize multiple technologies, and WHO-approved vaccines include two RNA, two protein subunit, four non-replicating viral vector, and three inactivated virus vaccines. Despite the incredible speed the vaccines were developed and administrated and the high vaccine efficacy of mRNA vaccines, the long-term control of the COVID-19 pandemic is challenging due to emerging immunoevasive viral variants.

2.3.1 Types of vaccines

All COVID-19 vaccines aim to induce immunological memory against SARS-CoV-2 without causing infection. The first COVID-19 vaccine candidates to enter phase-I trials were based on various technologies, including recombinant protein subunit, lipid-capsulated mRNA, DNA plasmids, and lentiviral and adenoviral vectors (Thanh Le et al., 2020). Later, virus-like particle (VLP) vaccines as well as attenuated and inactivated whole virus vaccines advanced into clinical trials. Various vaccine platforms offer flexibility in administration routes, manufacturing processes, and storage conditions. Intramuscularly administrated vaccines are traditionally the most common ones in preventing viral infections, and in the EU, all authorized COVID-19 vaccines are administrated through an intramuscular route (EMA, 2023). However, vaccines that are administrated through intranasal and subcutaneous routes are also developed. Especially intranasal vaccines are attractive since they could prevent the replication and transmission of SARS-CoV-2, as shown in hamsters and rhesus macaques (Deng et al., 2023; Hassan et al., 2021). However, whether humans are truly protected from SARS-CoV-2 after intranasal vaccination is unclear since one of the first COVID-19 intranasal vaccines, effective in animals, failed to induce a mucosal immune response in humans (Madhavan et al., 2022).

Subunit vaccines

Recombinant protein subunit vaccine technology is well established, and subunit vaccines have been approved against multiple viruses, including influenza, hepatitis B, and human papillomavirus (FDA, 2023b). Subunit vaccines are formulated by expressing virus protein or parts of the protein in cell culture and purifying the protein. High purity improves vaccine safety; however, subunit vaccines usually require the use of adjuvants to induce sufficient immune responses (Jidang Chen et al., 2021). For SARS-CoV-2, three adjuvanted subunit vaccines have been authorized by EMA, Nuvaxovid by Novavax, VidPrevtyn Beta by Sanofi Pasteur and Bimervax by HIPRA Human Health. Bimervax and VidPrevtyn Beta contain a part of the spike from Alpha and Beta variants, and both have been shown to induce higher antibody levels against the Omicron variant than the original Comirnaty vaccine when used as a booster dose (EMA, 2023).

mRNA and DNA vaccines

Nucleic acid vaccines, which contain either mRNA or DNA encoding a target antigen, have emerged as a promising approach to vaccination. Upon delivery, the mRNA or DNA is taken in by host cells which express and present the antigen to immune cells inducing a strong antibody and T cell-mediated immune responses (Jackson et al., 2020). One of the major advantages of nucleic acid vaccines is their ease of production and design. Compared to traditional vaccines, only a small amount of mRNA or DNA is required to manufacture nucleic acid vaccines. However, mRNA molecules are known to be unstable and therefore need to be encapsulated into lipid nanoparticles to protect them from degradation. Additionally, low storage temperatures are required to maintain their stability.

While the technology for mRNA vaccines has been studied since the 1980s, it was not until the COVID-19 pandemic that mRNA vaccines were authorized for human use. Since 2020, two COVID-19 mRNA vaccines encoding spike protein of original SARS-CoV-2 Wuhan-Hu-1 strain, Comirnaty and Spikevax, have been approved in US and EU (EMA, 2023). Over 90% of administered vaccine doses in both areas are mRNA vaccines. So far, Moderna has developed vaccines against the original Wuhan-Hu-1 strain and Beta and Omicron BA.1 and BA.4/5 variants, highlighting the ease of designing an mRNA vaccine.

Viral vector vaccines

Viral vector vaccines use unrelated viruses to deliver the genes encoding for target antigens into host cells, where they are produced and presented to T cells. Vector viruses are modified to reduce their virulence while maintaining their capability to

infect human cells. The most commonly used vector viruses are adenovirus and vesicular stomatitis virus (Travieso et al., 2022). Viral vectored vaccines can induce strong B and T cell immunity; however, responses against viral vector vaccines are decreased if pre-existing immunity against the vector virus exists. Most authorized COVID-19 viral vector vaccines use either human or chimpanzee adenovirus strains, which have been modified to prevent their replication. In EU, two adenoviral vector vaccines that include the spike gene have been approved, Jcovden by Janssen and Vaxzevria by Astra-Zeneca (EMA, 2023).

Virus-like particle vaccines

VLP vaccines are composed of one or more viral proteins that can self-assemble in the absence of a viral genome and non-structural proteins. VLP vaccines mimic the structure of the virus, and therefore they are thought to induce a more robust humoral immune response than protein subunit vaccines (Tariq et al., 2022). Before the COVID-19 pandemic, several VLP vaccines were approved in the US, including vaccines against human papillomavirus and hepatitis E virus (Dai et al., 2018). In May 2023, two COVID-19 VLP vaccines were in phase 3 clinical trials, and one of them was approved in Canada (McGill COVID19 Vaccine Tracker Team, 2022).

Inactivated and attenuated vaccines

Whole virus vaccines, including inactivated and attenuated vaccines, represent the oldest types of vaccines. Live attenuated vaccines involve the administration of viruses that have undergone a weakening process, while inactivated vaccines employ viruses that have been rendered chemically non-infectious, thereby ensuring enhanced safety. However, one drawback of inactivated vaccines is their potential to lose immunogenicity, and they often induce only humoral immune responses. Although growing viruses in cell cultures is relatively simple, an extensive amount of time is required, and the process can result in the introduction of mutations as viruses adapt to the host cells. Additionally, a concern with live attenuated whole virus vaccines against SARS-CoV-2 is the possibility of recombination between the attenuated vaccine strain and live circulating virus strain resulting in a novel infectious virus. In EU, one inactivated COVID-19 vaccine has been approved, Valneva by Valneva Austria (EMA, 2023).

2.3.2 Vaccine effectiveness

Vaccine effectiveness (VE) is a measure of protection against disease or other outcomes, such as hospitalization or death, in real-world communities. In contrast,

vaccine efficacy is measured during clinical trials and based on WHO, the vaccine should show at least a 50% efficacy rate to be approved (WHO, 2021). VE indicates the reduction in the number of people who will get the disease when they are exposed to the virus. VE is affected by study population factors, circulating virus variant, vaccine type, number of received vaccine doses, and time after the vaccination (Evans & Jewell, 2021). Different vaccine types can have different initial VE, and a vaccine with initially high VE can experience more rapid waning resulting in lower protection and a resurgence of infections months after vaccination (Burdin et al., 2017).

In clinical trials, Comirnaty vaccine showed a high efficacy of 95% in preventing symptomatic SARS-CoV-2 infection (Polack et al., 2020). During 2021, the VE of mRNA vaccines was estimated to be around 89–92% against symptomatic Alpha variant infection and 92–95% against symptomatic Delta infection. The VE was slightly higher against hospitalization or death (Nasreen et al., 2022). In another study, the VE of Comirnaty against symptomatic Alpha infection was 94% and against Delta 88% (Lopez Bernal et al., 2021). With COVID-19 vaccines the VE declines within months after vaccination and before the Omicron period, VE against symptomatic infection was estimated to drop by 20–30 percentage points in six months (Feikin et al., 2022). In comparison, influenza virus vaccines have a VE of around 50% against hospitalization (Ferdinands et al., 2019), and Ebola virus vaccine has a VE of nearly 100% (Henao-Restrepo et al., 2017).

2.3.3 Vaccinations in Finland

In Finland, COVID-19 vaccinations started at the end of December 2021 (Finnish Institute for Health and Welfare, 2023a). Healthcare workers treating COVID-19 patients were vaccinated first with a 21-day dose interval with the Comirnaty vaccine, followed by nursing home residents, staff, and persons with a predisposing condition for severe COVID-19. At the beginning of February 2021, the vaccination interval was increased to three months. Comirnaty, Spikevax, and Vaxzervia were all administered until the use of Vaxzervia was restricted in people under 65 years in March 2021 due to an increased risk of blood clotting related to the vaccine (Greinacher et al., 2021). The use of Vaxzevria ceased completely in November 2021.

In autumn 2021, the vaccine recommendations were modified to include a third vaccine dose of mRNA vaccine six months after the previous vaccine dose to all adults. However, under 30-year-old males received only Comirnaty due to a somewhat increased risk of myocarditis associated with Spikevax (Karlstad et al., 2022). The first protein subunit vaccine, Nuvaxovid, was authorized in December of

2021 (EMA, 2023) and was offered to all adults who did not want the mRNA vaccine as a third dose.

In September 2022, bivalent mRNA vaccines from Pfizer-BioNTech and Moderna were taken into use, and a fourth vaccine dose was recommended for adults aged 65 or older and those belonging to medical risk groups (Finnish Institute for Health and Welfare, 2022). Bivalent vaccines have mRNAs encoding for spike protein of the original SARS-CoV-2 and Omicron variant. Currently, three doses are recommended for adults under 60 years old, four doses for 60–64 years old, and seasonal booster doses for those over 65 years or those in medical risk groups. SARS-CoV-2 infection is considered as one vaccine dose. Finland had relatively low vaccine hesitancy; 84.5% of adults have received two vaccine doses, 65.2% of adults have received three doses, and 26.7% of adults have received four doses (Finnish Institute for Health and Welfare, 2023c). Worldwide 70% of the population has received at least one vaccine dose (https://ourworldindata.org/covid-vaccinations). According to the Finnish Institute for Health and Welfare (THL), vaccinations prevented over 7,000 COVID-19 deaths in Finland between December 2020 and March 2022, in addition to preventing deaths in which SARS-CoV-2 infections contributed indirectly (Finnish Institute for Health and Welfare, 2023b).

2.4 Adaptive immunity against SARS-CoV-2 and COVID-19 vaccination

Adaptive immunity is highly antigen-specific and specialized in eliminating microbes and preventing infections. Adaptive immune responses are activated in 5-10 days and function as a second line of defense after the faster activating innate immune system (Sego et al., 2020). Adaptive immunity consists of three fundamental cell types: B cells and CD4+ and CD8+ T cells. T cells require antigen presentation by antigen-presenting cells (APC) for expansion and effector functions. Activated CD4+ T cells costimulate B and CD8+ T cells, while activated CD8+ T cells destroy infected cells. B cell activation and differentiation leads to the production of affinity-maturated and isotype-switched antibodies that inactivate viruses outside of cells. T and B cells can differentiate into memory cells that respond to previously encountered microbes and form the basis of effective vaccinations against infectious diseases. SARS-CoV-2 infection and COVID-19 vaccinations are known to activate CD4+ and CD8+ T cells and induce the production of antibodies. Since most people in Finland are vaccinated with mRNA vaccines, this chapter will focus mainly on mRNA vaccines and infection-induced responses.

2.4.1 Antibody responses in COVID-19 patients

In SARS-CoV-2 infection as well as in other viral infections, B cells are activated when viral antigen binds to their B cell receptor located on the surface of the cell (Qi et al., 2022). With the help of CD4+ T cells, B cells multiply and differentiate first into the germinal centre (GC) B cells and short-lived plasma cells that initially produce antigen-specific immunoglobulin (Ig) M antibodies, followed by IgG and IgA antibodies. Next, GC B cells migrate to B cell follicles of secondary lymphoid tissue to form germinal centres and differentiate into long-lived antibody-secreting plasma cells and memory B cells. During the germinal centre response, antibodies can class switch to different Ig types and accumulate somatic mutations, which increase antibody affinity against the target antigen.

Natural SARS-CoV-2 infection induces the production of serum IgM, IgG, and IgA antibodies against a range of viral proteins, and N, S, ORF3b, and ORF8 proteins elicit the strongest SARS-CoV-2-specific antibody response (Hachim et al., 2020; Long et al., 2020). Serum IgM, IgG, and IgA antibodies are produced 2 to 16 days after symptom onset or after the first positive RT-qPCR test result, and almost all patients seroconvert within two weeks (Kellam & Barclay, 2020; Long et al., 2020; H. Ma et al., 2020; Suthar et al., 2020). IgM antibodies are mostly detected before IgG antibodies, although in some studies, IgM antibodies were detected simultaneously or after the secretion of IgG (Kaplonek et al., 2023; Long et al., 2020; Qu et al., 2020). The levels of IgG antibodies continue to increase after IgM antibody levels have reached their peak two weeks after symptom onset (Sun et al., 2020). IgM antibody titers decline rapidly one to six months post-infection, while IgG antibodies are sustained for at least a year after infection (H. Wang et al., 2021). IgG expression kinetics suggest successful recruitment of long-lived plasma cells, which migrate to reside in the bone marrow where they continue to produce antibodies for months or years. Furthermore, anti-S antibodies are sustained for a longer period of time than anti-N antibodies (Lumley et al., 2021), and studies suggest that anti-N IgG antibodies have a half-life of 71–85 days, while anti-S antibodies have a half-life of 184–229 days (Lumley et al., 2021; Wei et al., 2021; Wheatley, Juno, et al., 2021).

IgG is the most abundant antibody in the blood, while in the mucus epithelium of intestinal and upper respiratory tracts, secretory IgA is the main isotype (Janeway CA Jr, Travers P, Walport M, 2001). Secretory IgA prevents the attachment of viruses to epithelial cells and likely offers protection against respiratory virus infection, as has been demonstrated with influenza A and B viruses (Asahi-Ozaki et al., 2004; Okamoto et al., 2009). In SARS-CoV-2 infection, IgG and IgA are detected in saliva and nasal fluids, although the responses vary significantly between patients (Cervia et al., 2021; Isho et al., 2020). A study analyzing saliva antibody responses in SARS-CoV-2 infected children detected higher anti-S antibodies in asymptomatic infections

compared to symptomatic, suggesting that mucosal antibodies may prevent systemic infection (Dobaño et al., 2021). This finding is supported by a study reporting that dimeric secretory IgA neutralizes SARS-CoV-2, and the neutralization is more potent than monomeric serum IgA neutralization (Z. Wang, Lorenzi, et al., 2021).

Spike-specific antibodies are mostly neutralizing antibodies that block the virus entry into host cells (Brouwer et al., 2020). Neutralizing antibodies develop at the same time as other antibodies, and they correlate well with anti-S antibodies (Suthar et al., 2020). In one study, RBD-specific neutralizing antibodies were noted to have low levels of somatic hypermutation when isolated at the early convalescent phase (Gaebler et al., 2021; Muecksch et al., 2021; Rogers et al., 2020). Although somatic hypermutations increased later, the results suggest that neutralizing antibodies do not require high affinity and are quickly produced. Neutralizing antibodies have been shown to correlate with protective immunity against SARS-CoV-2 re-infection (Chandrashekar et al., 2020; Imai et al., 2020; Khoury et al., 2021); however, protective antibody levels have not been determined against SARS-CoV-2, like against influenza A virus (Tsang et al., 2014).

Despite the importance of virus-specific antibodies in protection, seroconversion after the first SARS-CoV-2 infection is not associated with more favorable disease outcomes (X. Chen et al., 2020). Studies have shown that patients with more severe diseases have higher viral load and consequently higher serum antibody levels (Röltgen et al., 2020; Wei et al., 2021). Neutralizing antibodies alone cannot clear an already established infection, which is supported by the recovery of patients with antibody deficiencies from SARS-CoV-2 infection (Soresina et al., 2020).

Pre-existing antibodies against seasonal hCoVs have been proposed to offer some protection against SARS-CoV-2 (Galipeau et al., 2021). A study with US samples found higher levels of OC43 RBD-specific antibodies in recovered patients compared to deceased patients (Kaplonek et al., 2023). Another study of sub-Saharan Africa samples found cross-reactive S-specific antibodies to SARS-CoV-2 from 229E and NL63 infected, but not from OC43 infected individuals (Tso et al., 2021). However, a few studies have found no or very little cross-neutralization of SARS-CoV-2 (Dhochak et al., 2022; Galipeau et al., 2021; Wells et al., 2022), and since early neutralizing antibodies lack somatic hypermutations, they are potentially developed from naïve B cells or other B cell subsets with little affinity maturation such as B-1 cells (Sette & Crotty, 2021).

2.4.2 Antibody responses after COVID-19 vaccination

COVID-19 mRNA and adenoviral vector vaccines used in Finland induce antibody responses against SARS-CoV-2 S protein but not against N protein. Similar to infection, vaccinations induce the production of IgG antibodies and some IgA and

IgM antibodies in serum; however, mucosal antibodies are not induced (Terreri et al., 2022). Antibodies are developed 14–30 days post mRNA vaccination, and the peak antibody levels are reached within four weeks (Goel et al., 2021; Payne et al., 2021; Sahin et al., 2021). In clinical trials, the completion of the primary mRNA vaccine series resulted in neutralizing antibody response in all vaccinated participants (Walsh et al., 2020). Peak antibody levels and seropositivity are higher in the younger population than those 65 or older or immunosuppressed (Ward et al., 2022).

Vaccine-induced antibodies remain detectable for six to eight months (Korosec et al., 2022), and the half-life of neutralizing antibodies is estimated to be 66-182 days, which resembles antibody durability after SARS-CoV-2 infection (Doria-Rose et al., 2021). Further immunizations can rescue decreasing antibody levels, and third and fourth vaccine doses have been shown to increase neutralizing antibodies (Regev-Yochay et al., 2022). A systemic review reported that SARS-CoV-2 infected patients who have also been vaccinated have more durable antibody responses compared to only vaccinated or infected individuals (Bobrovitz et al., 2023).

Vaccine-induced antibody responses are challenged by evolving immunoevasive variants. Especially Beta, Gamma, Delta, and Omicron variants exhibit marked reductions in neutralization efficacy when compared to the original SARS-CoV-2 strain (Lustig et al., 2021; Supasa et al., 2021; Tada et al., 2022). However, emerging evidence suggests that vaccine-induced anti-RBD antibodies demonstrate enhanced cross-neutralization capabilities against diverse variants, surpassing the neutralizing potential of infection-induced antibodies (Röltgen et al., 2022). The observed disparity in neutralizing efficacy may diminish when evaluating antibody responses a few months after infection, as infection-induced antibody responses require adequate time to mature. Furthermore, the breakthrough infection in vaccinated individuals elicits the production of highly potent neutralizing antibodies, surpassing those generated by natural infection or vaccination alone, thereby aiding in the control of emerging immunoevasive variants (H. Yu et al., 2023).

Earlier SARS-CoV-2 infections and vaccinations can potentially result in immune imprinting (Wheatley, Fox, et al., 2021). Immune imprinting or original antigenic sin is a common phenomenon with influenza infections, where re-infections with antigenically different influenza viruses boost antibodies against those epitopes that are shared with the previously infecting virus strain, leading to low-affinity antibodies against new viral epitopes (Krammer, 2019). With SARS-CoV-2, boosting with a bivalent booster vaccine has been shown to induce good levels of Omicron neutralizing antibodies; however, antibodies neutralized the original SARS-CoV-2 virus even better than Omicron variants and original monovalent vaccine induced comparable responses as bivalent vaccine (A. Y. Collier et al., 2023). Similar results were also seen in Omicron-infected patients who

were previously vaccinated with the original Wuhan-Hu-1 strain and who recalled mainly cross-reactive B cells formed in earlier vaccinations (Cao et al., 2023). Cao et al. did not detect the expansion of new B cell clones specific only to Omicron; however, booster vaccination with a bivalent vaccine has been shown to recruit de novo B cells targeting variant epitopes (Alsoussi et al., 2023).

2.4.3 Memory B cells

Memory B cells are formed during GC response and survive long-term in the circulation for immune surveillance (Akkaya et al., 2020). Memory B cells do not produce antibodies, but when re-encountering antigen, they activate and differentiate into plasma cells producing high-affinity antibodies. Both SARS-CoV-2 infection and COVID-19 vaccinations produce memory B cells (Goel et al., 2021) that increase in numbers in the following months (Terreri et al., 2022; Wheatley, Juno, et al., 2021). Memory B cells have been shown to persist at high concentrations at least 12 months post-infection (K. W. Cohen et al., 2021; Jernej et al., 2022) and nine months post-vaccination (Terreri et al., 2022). In SARS-infected patients, memory B cells have become undetectable in 3-6 years (Tang et al., 2011) and similar waning is possible for SARS-CoV-2-specific memory B cells. However, re-immunization with SARS-CoV-2 occurs repeatedly, which might change memory B cell durability.

In re-activation, new memory B cells are formed. Additional COVID-19 vaccine doses have been shown to induce germinal centre reactions and secondary affinity maturation, further improving the quality of the antibody responses (Alsoussi et al., 2023). Furthermore, SARS-CoV-2 convalescent patients who have subsequently been vaccinated have memory B cell pools which expand and mature into high-affinity neutralizing clones without the loss of previous memory B cell pools (Sokal et al., 2021).

2.4.4 T cell responses

Naive T cells are activated and differentiate into effector cells when they receive suitable costimulatory signals and are presented by their specific antigen in the form of peptides. Intracellular pathogen peptides are presented to CD8+ T cells through major histocompatibility complex (MHC) class I molecules on the surface of infected cells, and pathogens ingested by APCs, including dendritic cells and macrophages, are presented to CD4+ T cells through MHC class II molecules. After clearance of infection, a small proportion of effector T cells are maintained as memory T cells, capable of responding to re-infections (Kaech et al., 2002). CD4+ T cells are important for a robust CD8+ T cell response. For instance, in influenza

infection, CD4+ T cells have been shown to help memory CD8+ T cells to migrate into lung airways and recruit more CD8+ T cells from circulation (Laidlaw et al., 2014, 2016).

SARS-CoV-2 infection has been shown to activate both CD4+ and CD8+ T cells, and SARS-CoV-2-specific T cell responses have been detected 2-10 days post symptom onset (Kundu et al., 2022; A. T. Tan et al., 2021; Weiskopf et al., 2020). SARS-CoV-2 S-specific CD4+ T cell responses are induced in almost all COVID-19 patients, while CD8+ T cell responses are less prominent and present in around 70% of infected individuals (Grifoni et al., 2020; Le Bert et al., 2020). CD4+ and CD8+ T cells can also target M and N proteins, and to a lesser extent nsp3, nsp4, nsp12, and ORF3a proteins (Tarke et al., 2021), although S protein is recognized most consistently (Grifoni et al., 2020). After COVID-19 mRNA vaccination, a similar magnitude of S-specific CD4+ and CD8+ T cell responses are detected than after infection (Sahin et al., 2021). A recent study demonstrated that vaccinated individuals with a breakthrough infection could activate CD8+ T cells specific to other proteins than spike, eliciting a broader repertoire of T cells and indicating that vaccination does not prevent the future recruitment of naïve T cells (Minervina et al., 2022).

SARS-CoV-2-specific T cell responses are associated with milder disease forms (Rydyznski Moderbacher et al., 2020; Sekine et al., 2020). In animal models, CD4+ T cells protect mice from lethal SARS and MERS infection (J. Zhao et al., 2016) and CD8+ T cells reduced viral loads in rhesus macaques with breakthrough SARS-CoV-2 infection even with low neutralizing antibody levels (McMahan et al., 2021). This is further supported by studies in patients with antibody deficiencies or cancer patients receiving B cell depleting therapy who recover from COVID-19 (Bange et al., 2021; Soresina et al., 2020). T cell numbers, expression of effector cytokine tumor necrosis factor alpha (TNFα), and low expression of exhaustion marker PD-1, are associated with improved outcomes (Zeyu Chen & John Wherry, 2020; Su et al., 2020). The severe disease is characterized by high expression of PD-1 and CD8+ T cell effector molecules perforin and granzyme B, which may contribute to immunopathology (Mathew et al., 2020; Zenarruzabeitia et al., 2021). Of note, T cells express PD-1 at acute phase of the disease and PD-1+ cells are shown to be functional and continue to circulate (Minervina et al., 2022; Rha et al., 2021), indicating that expression of PD-1 is a sign of highly activated T cells rather than a marker of exhaustion.

SARS-CoV-2-specific memory CD4+ T cells have been shown to be more abundant than memory CD8+ T cells (K. W. Cohen et al., 2021). Circulating memory T cells are maintained for at least nine months after infection (Wirsching et al., 2022) and six months after vaccination (Maringer et al., 2023). With SARS, circulating memory T cells have been detected 17 years after infection, indicating

long-lasting protection from severe SARS-related diseases (Le Bert et al., 2020). In addition to circulating memory T cells, some memory T cells persist long-term in epithelial tissues. Studies of tissue-resident memory T cells are more difficult than circulating T cells, although important, since memory CD4+ and CD8+ T cells in the airway of mice mediate the clearance of SARS and MERS infections (J. Zhao et al., 2016). In addition, a study found that vaccinated individuals with breakthrough infection had nasal-resident CD4+ and CD8+ T cells, which recognized S and other SARS-CoV-2 epitopes and persisted for at least four months (Lim et al., 2022).

CD4+ T cells can differentiate into multiple subtypes, including T helper 1 (Th1) and Th2 effector cells. Th1-type response is generally considered to direct CD8+ T cell and innate immune responses and protect against intracellular bacteria and virus infections (McKinstry et al., 2012), while Th2-type response is linked with antibody production and eradication of extracellular pathogens (Corripio-Miyar et al., 2022). In SARS-CoV-2 infection and vaccination, the polarization of the response seems to be of Th1 type with high production of interferon gamma (IFN-γ) and no interleukin 4 (IL-4) (Grifoni et al., 2020; Rydyznski Moderbacher et al., 2020; Sahin et al., 2021), suggesting the effective recruitment of cytotoxic CD8+ T cells. Induction of inappropriate CD4+ T cell subtype can lead to poor protection against intracellular viruses, and also to enhanced disease, as shown in SARS-vaccinated mice which elicited Th2 type immunopathology and massive eosinophil infiltration when challenged with infectious virus (Tseng et al., 2012). Therefore, vaccines need to be designed and studied carefully to avoid immunopathology or delays in virus clearance.

Pre-existing hCoV-specific T cells that cross-react with SARS-CoV-2 have been found in samples collected before the COVID-19 pandemic. Depending on the study, 20-80% of people have been detected to have cross-reactive memory CD4+ T cells (Grifoni et al., 2020; Le Bert et al., 2020; Sekine et al., 2020), while cross-reactive CD8+ T cells are observed less frequently. Cross-reactive CD4+ T cells target structural proteins and nsp epitopes (Grifoni et al., 2020; Kundu et al., 2022). Although there is no evidence that these pre-existing clones would be expanded over de novo clones in SARS-CoV-2 infection, a study found higher frequencies of cross-reactive T cells in PCR-negative individuals exposed to SARS-CoV-2 compared to PCR-positive individuals (Kundu et al., 2022), suggesting that pre-existing T cells have a role in controlling the infection.

The evaluation of both humoral and cellular immune responses is crucial for assessing vaccine effectiveness and long-term protection. However, SARS-CoV-2 adaptive immune responses are defined by marked heterogeneity between individuals, and B and T cell responses are affected by the MHC molecule and B and T cell receptor variability, innate immune responses, pre-existing cross-reactive B and T cells, localization and magnitude of the infection, and viral antigen

production (Sette & Crotty, 2021). Despite individual variation, more research is needed to understand the durability of memory B and T cell responses upon repeated breakthrough infections and the role of adaptive immunity in providing sustained protection against SARS-CoV-2 variants at the population level.

3 Aims

The overall aim of this study was to develop assays to measure humoral and cell-mediated immunity and analyse COVID-19 vaccine and SARS-CoV-2 infection-induced humoral and cell-mediated responses in Finnish healthcare workers and COVID-19 patients.

The specific research objectives were:

1. To set up and optimize a serological assay for measuring the SARS-CoV-2-specific antibody levels and characterize the cross-reactivity of antibodies against other human coronaviruses.
2. To study the induction of neutralizing antibodies after COVID-19 mRNA vaccination and analyse immunity against SARS-CoV-2 variants of concern circulating in Finland during 2020 to summer of 2021.
3. To follow-up the longevity of COVID-19 mRNA vaccine-induced humoral immunity.
4. To compare the COVID-19 vaccine and infection-induced humoral and cell-mediated immune responses and to measure the sensitivity of T cell responses to the antigenic variation in the viral spike glycoprotein.

4 Materials and Methods

4.1 Study participants

COVID-19 patients (I, II, IV)

In studies I, II, and IV, PCR-confirmed COVID-19 patients were recruited from Turku University Hospital (TYKS, Turku, Finland) or Helsinki University Hospital (HUS, Helsinki, Finland). Serum samples were collected during the acute phase (0–13 days after symptom onset or positive PCR test result when the date of the symptom onset was not available) and convalescent phase (>14 days) of the disease. Study I included 101 patients from TYKS and HUS aged 24–86 (mean 54 years; 49 females and 52 males) with 92 acute phase and 27 convalescent phase serum samples (17 paired serum samples). In study I, serum samples were inactivated at 56°C for 30 minutes. Study II included 50 home-treated patients from HUS aged 19–93 years (mean 43 years; 33 females and 17 males) with 50 convalescent phase serum samples. Study IV included 15 patients from TYKS aged 32–78 years (mean 53 years; six females and nine males) with convalescent phase serum samples and peripheral blood mononuclear cells (PBMCs) collected.

Negative control participants (I-IV)

In studies I-III, COVID-19 negative control samples were selected randomly from a pool of de-identified serum samples collected at TYKS for epidemiological purposes before the COVID-19 pandemic. Study I included 100, study II 20, and study III 30 negative control samples. In study IV, serum samples and PBMCs were collected from 13 non-vaccinated control participants with no previous SARS-CoV-2 infection.

COVID-19 vaccinated health care workers (II-IV)

In studies II-IV, healthcare workers (HCWs) were recruited among personnel of TYKS and HUS. HCWs received two doses of the BNT162b2 mRNA vaccine (Pfizer-BioNTech) with three-week dose intervals as part of occupational healthcare.

In study II, serum samples were collected from 180 HCWs aged 20–65 years (mean 43 years; 149 females and 31 males) before or on the day of the first vaccine dose and three weeks after the first and second vaccine doses. In study III, follow-up was continued for 52 HCWs aged 22–65 years (mean age 45 years; 35 females and 17 males), selected randomly from the larger cohort of study I, and serum samples were collected three and six months after the second vaccine dose. In study IV, in addition to serum samples, PBMCs were collected from 23 HCWs working at TYKS three weeks and three and six months after the second vaccine dose. In study IV, the participants were 26–60 years (mean 39 years; 20 females and three males). All participants were asked to fill out a study questionnaire at each visit. Immunocompromised patients were excluded from the study.

4.2 Ethics

All study participants provided a written informed consent. The Ethics Committees of the Southwest Finland health district and the Helsinki-Uusimaa health district approved the study protocols. Decision numbers are for TYKS HCWs ETMK 19/1801/2020 (EudraCT 2021-004419-14), for HUS HCWs, negative control participants (IV), and COVID-19 patients (IV) HUS/1238/2020 (EudraCT 2021-004016-26), and study I COVID-19 patients HUS/32/2018.

4.3 Expression and purification of proteins

In study I, synthetic genes encoding the N proteins of human coronaviruses (SARS-CoV-2, SARS, MERS, HKU1, OC43, 229E, and NL63) were obtained from GeneArt, and codon-optimized genes encoding the SARS-CoV-2 S1 (aa residues 16–541) and RBD (aa residues 319–541) proteins were obtained from GeneUniversal (Table 1). N protein encoding genes were cloned into a pBVboost vector with N-terminal glutathione-S-transferase (GST), and S1 and RBD genes were cloned into a mammalian expression vector with C-terminal polyhistidine tail and mouse IgG2a Fc part (later referred as mFc-8xHis). N proteins were produced in *Spodoptera frugiperda* (Sf-9) cells (Airenne et al., 2009) and purified with Glutathione Sepharose® 4B (Merck). S1 and RBD -proteins were produced in human embryonic kidney (HEK293F) cells and purified with Protino Ni-NTA column (Macherey-Nagel). Protein purity was evaluated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Page Blue staining. Protein concentration was measured with Nanodrop or by running known amounts of BSA standard in SDS-PAGE. In study I, for negative control antigens, a mouse myostatin growth factor proregion (proMstn; aa residues 1–263 with a D76A stabilizing mutation; GenBank accession AAI05675) fused to a GSGGGG linker and mouse IgG2a Fc region tagged

with polyhistidine tail (proMstn-mFc-6xHis), and GST were expressed as described above. In study II-IV, recombinant SARS-CoV-2 GST-N produced in Sf-9 cells and S1-mFc-8xHis produced in HEK293F cells were used.

Table 1. Genbank accesion numbers for human coronavirus proteins.

Virus	Strain	Proteins	Genbank accession number
SARS-CoV-2	Wuhan-Hu-1	N	NC_045512.2
	Wuhan-Hu-1	S1 and RBD	MN908947.3
SARS	HKU-39849	N	AY278491.2
MERS	EMC/2012	N	JX869059.2
HKU1	N09-1605B	N	KY674943.1
OC43	HCoV_OC43/Seattle/USA/SC9430/2018	N	MN306053.1
229E	HCoV_229E/Seattle/USA/SC379/2016	N	KY621348.1
NL63	N06-1144B	N	KY554967.1

4.4 Enzyme immunoassay (EIA)

In study I, 96-well microtiter plates (Nunc Maxisorp, Thermo Fisher Scientific) were coated with purified recombinant GST-N, RBD-8xHis, RBD-mFc-8xHis, S1-8xHis, and S1-mFc-8xHis proteins. Corresponding molar amounts of GST and proMstn-mFc-6xHis proteins were used as negative control antigens. The next day, the plates were washed once and incubated with 1:300 diluted serum samples in assay buffer (5% swine serum [Biological Industries] in PBS-Tween20) for two hours at 37°C. Plates were washed, and incubated with horseradish peroxidase-labeled anti-human IgG (Dako), anti-human IgA (Invitrogen), or anti-human IgM (Dako) antibodies for one hour at 37°C. After washing four times, wells were incubated with TMB One substrate (3, 3', 5, 5'-tetramethylbenzidine, Kementec Solutions) for 20 min, and the reaction was stopped with 0.1 M H_2SO_4. The absorbance was measured at 450 nm wavelength with a Victor Nivo plate reader (PerkinElmer).

In studies II-IV, EIAs were performed as in study I with minor modifications. Plates were coated only with SARS-CoV-2 GST-N and S1-mFc-8xHis proteins, and before the addition of serum samples, the plates were blocked with assay buffer. In studies III and IV, IgM and IgA antibodies were not measured.

Conversion to units

In all studies, negative and positive control pools were included in each plate. The negative pool consisted of SARS-CoV-2 negative serum samples collected from one

to three years old children (Kazakova et al., 2019). The positive pool consisted of COVID-19 patient serum samples positive for SARS-CoV-2 N and S1 proteins from study I; however, individual samples in the positive pool varied in each study due to the low volume of the samples. In study I, the absorbance for the negative control antigen (GST or proMstn-mFc-6xHis) was subtracted from the respective sample absorbance. In all studies, optical density (OD) values were converted into EIA units using linear interpolation between positive (marked as 100 units) and negative (marked as 0 units) control pools. In studies II-IV, units <1 were marked as 1 unit. In study I, the unit values for SARS-CoV-2 N–based EIA were adopted to other hCoV EIAs because no confirmed negative and highly positive control samples were available for SARS, MERS, and seasonal coronaviruses.

Cut-off values

In study I, the cut-off units were determined with receiver operating characteristics (ROC) analysis by choosing 98–100% specificity for anti-SARS-CoV-2 N, S1, and RBD IgG, IgA, and IgM EIAs. In study II–IV, the cut-off units for S1-based EIA were calculated as the average of negative serum samples plus three or six standard deviations (SDs).

4.5 Viruses, virus culture and sequencing

In studies I-III, SARS-CoV-2 variants were isolated from COVID-19 patient nasopharyngeal samples in a biosafety level 3 (BSL3) laboratory in THL or University of Helsinki (Table 2). Isolates were grown in infection media (DMEM or EMEM with 2% fetal bovine serum (FBS), 2mM L-glutamine, and penicillin/streptomycin) using African green monkey kidney epithelial (VeroE6) cells (ATCC) or VeroE6 cells expressing TMPRSS2 (VeroE6-TMPRSS2-H10) kindly provided by Dr Jussi Hepojoki, University of Helsinki (Rusanen et al., 2021). Supernatants containing viruses were harvested, cell debris was removed, and aliquots were stored at -80 °C. Fifty-percent tissue culture infective dose ($TCID_{50}$) of virus stocks was determined with endpoint dilution assay: ten-fold virus dilutions in infection media were applied onto 96-well tissue culture plates (Sarstedt) containing 50,000 VeroE6 (study I) or VeroE6-TMPRSS2-H10 (study II and III) cells per well, and the plates were incubated for three days at 37°C and 5% CO_2. Cells were fixed with 4% formaldehyde and stained with crystal violet. $TCID_{50}$ was calculated with the Reed-Muench method.

In studies II and III, the viral RNA was extracted from virus stocks using the RNeasy Mini kit (Qiagen), and sequencing was performed by Dr. Teemu Smura at University of Helsinki. Whole genome sequences of SARS-CoV-2 isolates used in

studies II and III were deposited in GenBank. Pango lineage and aa changes were identified with Nextclade (https://clades.nextstrain.org/).

Table 2. SARS-CoV-2 isolates used in studies I-III.

Study	Virus isolate	Sample collection	Pango lineage (WHO label)	Genbank ID for virus stock	GISAID (EPI ISL)	Number of passages (p)	From
I, III	FIN-1-20	01/2020	B	MZ934691	407079	p5 in VE6	THL
II, III	FIN-25	02/2020	B.1	MW717675.1	412971	p3 in VE6 + p2 in VE6-H10	THL
II	SR-121	10/2020	B.1.463	MW717676.1	NA	p3 in VE6-H10	UH
II	85HEL	12/2020	B.1.1.7 (Alpha)	MW717677.1	NA	p3 in VE6-H10	UH
III	FIN35-21	01/2021	B.1.1.7 (Alpha)	OK448478.1	2589882	p4 in VE6-H10	THL
II	HEL12-102	01/2021	B.1.351 (Beta)	MW717678.1	NA	p3 in VE6-H10	UH
III	FIN32-21	01/2021	B.1.351 (Beta)	OK448476.1	3471851	p5 in VE6-H10	THL
III	FIN33-21	01/2021	B.1.525 (Eta)	OK638135	3471854	p5 in VE6-H10	THL
III	FIN37-21	05/2021	B.1.617.2 (Delta)	OK626882.1	2557176	p3 in VE6-H10	THL

VE6, VeroE6 cells; VE6-H10, VeroE6-TMPRRS2-H10 cells; UH, University of Helsinki; THL, Finnish Institute for Health and Welfare; NA, not available

4.6 Microneutralization test (MNT)

In studies I-III, the neutralization of SARS-CoV-2 variants by serum samples collected from infected or vaccinated individuals were measured using a microneutralization test (MNT). In study I, serum samples were serially diluted two-fold, starting at 1:5 dilution in infection media and incubated with an equal volume of 1,000 $TCID_{50}$ of SARS-CoV-2 isolate in 96-well tissue culture plates (Sarstedt) for one hour at 37 °C (final serum dilution 1:10) in BSL3 laboratory. VeroE6 cells were added (50,000 cells/well), and the plates were incubated at 37 °C and 5% CO_2 for three days. To visualize the cell death, cells were fixed with 4% formaldehyde and stained with crystal violet. Serum dilutions were done in triplicates, and neutralization titers were calculated as reciprocal of the serum dilution that inhibited the infection in at least two parallel wells.

In studies II and III, MNTs were performed as in study I with some modifications: serum dilutions started from 1:10, and samples were incubated with

an equal volume of 50 $TCID_{50}$ of SARS-CoV-2 isolates (final serum dilution 1:20). Instead of VeroE6 cells, 50,000 VeroE6-TMPRSS2-H10 cells per well were used. Serum dilutions were done in duplicates, and an inhibitory dose of 50% (ID50) was calculated as the reciprocal dilution inhibiting 50% of cell death. For geometric mean calculations neutralization titres <20 were marked as 10.

4.7 PBMC isolation and stimulations

PBMCs were isolated using Ficoll-Paque PLUS (GE Healthcare) density gradient centrifugation according to the manufacturer's instructions. After isolation, the PBMCs were counted and suspended in freezing media containing 10% dimethyl sulfoxide (DMSO) and 10% human AB serum (Sigma-Aldrich) and gradually frozen to -135°C until further use.

For PBMC stimulation assays, cryopreserved PBMCs were rapidly thawed and washed with culture media (RPMI-1640 (Gibco) supplemented with 10% human AB serum, 2mM L-glutamine, and penicillin/streptomycin). PBMCs were rested for eight hours and after resting, PBMCs were pelleted and resuspended into fresh culture media and counted. To activate SARS-CoV-2 S-specific T cells, $1x10^6$ live cells were plated into 96 well U-bottom plates (Thermo Fisher Scientific) and stimulated with peptide pools spanning the whole SARS-CoV-2 S protein (PepMiX, JPT Peptide Technologies, Table 3) at a 0.5 µg/ml final concentration in culture media for 48 hours. Peptide pools consisted of 15mers with 11mer overlaps. Ten µg/ml of purified tetanus toxoid (AJ Vaccines) and 0.4% of DMSO in culture media were used as positive and negative controls, respectively. Timepoints from the same individual were always processed simultaneously.

Table 3. SARS-CoV-2 peptide pools (PepMix™ from JPT Peptide) covering the whole spike protein.

Pango lineage / WHO label	Cat#	Number of aa changes*
B	PM-WCPV-S-1	-
B.1.1.7 / Alpha	PM-SARS2-SMUT01-1	10
B.1.351 / Beta	PM-SARS2-SMUT02-1	10
P.1 / Gamma	PM-SARS2-SMUT03-1	12
B.1.617.2 / Delta	PM-SARS2-SMUT06-1	10

*compared to the spike protein of the original Wuhan-Hu-1 strain

4.8 Flow cytometry

Stimulated PBMCs were washed with PBS, and dead cells were stained with Zombie Green dye (BioLegend) according to the manufacturer's instructions. PBMCs were subsequently stained with fluorescently labeled surface antibodies recognizing CD45, CD3, CD4, CD8, CD69, CD134, and CD137 (Table 4) in PBS containing 2% FBS for 30 min on ice. Stained PBMCs were washed and resuspended in PBS, and T cell subtypes were characterized with NovoCyte Quanteon Flow Cytometer (Agilent Technologies Inc) and analysed with NovoExpress v1.5.0 (Agilent Technologies Inc). A stimulation index (SI) was calculated by dividing the percentage of CD69+CD134+ or CD69+CD137+ cells after SARS-CoV-2 S peptide pool stimulation by the percentage of CD69+CD134+ or CD69+CD137+ cells after DMSO stimulation. Low-quality samples (CD3 cell count <10,000 cells/sample or missing CD4+ response to tetanus toxoid SI <2.0) were excluded.

Table 4. Fluorochrome labeled antibodies in FACS.

Antibody	Fluorochrome	Manufacturer	Cat#
Anti-human CD45	APC-eFluor780	Invitrogen/Life technologies	47-0459-42
Anti-human CD3	eFluor506	Invitrogen/Life technologies	69-0038-42
Anti-human CD4	eFluor450	Invitrogen/Life technologies	48-0049-42
Anti-human CD8a	PerCP-eFluor710	Invitrogen/Life technologies	46-0087-42
Anti-human CD69	PE	BD Biosciences	555531
Anti-human CD134	PE/Cyanine7	BioLegend	350012
Anti-human CD137	APC	BioLegend	309810

4.9 Luminex

The levels of secreted cytokines and effector molecules (IFN-γ, IL-2, TNF-α, and perforin) were analysed in THL with MILLIPLEX MAP Kit HCD8MAG-15K (Millipore) from the supernatant of stimulated PBMCs. The fluorescence of the samples was measured with Luminex MAGPIX magnetic bead analyser (Luminex Corporation), and the concentration was calculated from the median fluorescent intensity of seven diluted standards using a 5-parameter logistic regression. The samples with <35 beads/well were excluded from the final analysis. Samples that had a value from DMSO stimulation and either from tetanus or SARS-CoV-2 spike peptide pool stimulation were included in the final analysis.

4.10 Statistical analysis

In studies I–IV, data were analysed with GraphPad Prism version 8. Paired samples were tested with Wilcoxon signed-rank test, and unpaired samples were tested with Mann-Whitney U-test. In study I, statistical differences between multiple groups were analysed with one-way ANOVA followed by Tukey's multiple comparisons test. All tests were two-sided, and p-values <0.05 were considered statistically significant. Correlations were analysed with Spearman's correlation test.

5 Results

5.1 Development of SARS-CoV-2 serological assays (I)

In study I, enzyme immunoassay (EIA) was set up to detect various immunoglobulin classes against a combination of in-house-produced recombinant SARS-CoV-2 proteins. Antibody binding to S1 and RBD proteins with or without mFc tag was tested with six COVID-19 PCR-positive serum samples and six negative control samples. S1-mFc and RBD-mFc, presenting S1 and RBD in dimer, showed better antibody binding compared to monomeric S1 and RBD, and S1 showed somewhat higher signals than RBD. S1-mFc and RBD-mFc were selected for further studies and are here on referred to as S1 and RBD.

The performance of recombinant S1, RBD, and N protein-based EIA was tested with 119 serum samples collected from 101 PCR-confirmed COVID-19 patients at acute (1–13 days) and convalescent-phase (>14 days) of the disease. ROC curve analysis revealed area under the curve (AUC) values of 0.83, 0.92, and 0.86 for S1, RBD, and N protein-based IgG EIAs, respectively. The AUC values for IgA and IgM measurement were similar (anti-S1 IgA 0.86 and IgM 0.79; anti-RBD IgA 0.93 and IgM 0.91; anti-N IgA 0.86 and IgM 0.83). When the cut-off values were selected to be 8.5, 9.5, and 7.5 units, the specificities were 98%, 99%, and 99% for S1, RBD, and N protein-based IgG EIAs, respectively. IgA and IgM EIAs had specificities of 99–100%. The highest analytical sensitivity was with anti-N IgG (65% at acute phase; 96% at convalescent phase) and anti-RBD IgA (65% at acute phase; 96% at convalescent phase) measurement (Table 5). The combination of these two further increased the sensitivity (77% at acute phase; 100% at convalescent phase), indicating that the antibody kinetics vary between patients and that both N and RBD are needed for accurate detection of past SARS-CoV-2 infections.

SARS-CoV-2 infection-induced S1-specific IgG, IgM, and IgA antibody levels correlated well with neutralization titers against the Wuhan-Hu-1-like SARS-CoV-2 isolate (FIN-1-20, Spearman $r=0.865$, $r=0.860$, and $r=0.825$, respectively, $p<0.0001$). In addition, neutralization titers correlated with N-specific IgG ($r=0.786$, $p<0.0001$) but only moderately with N-specific IgM and IgA ($r=0.627$ and $r=0.646$, respectively, $p<0.0001$).

Table 5. Sensitivities (%) of SARS-CoV-2 N, S1, and RBD-based EIAs. Modified from Original publication I.

		N	S1	RBD	N AND/OR S1	N AND/OR RBD
COVID-19 PATIENTS, ACUTE PHASE (N=92)	IgG	65.2	41.3	48.9	67.4	67.4
	IgA	63.0	56.5	65.2	69.6	75.0
	IgM	51.1	48.9	57.6	57.6	65.2
	IgG/IgA	68.5	57.6	69.6	71.7	77.2
	IgG/IgM	66.3	51.1	63.0	71.7	72.8
	IgA/IgM	66.3	57.6	73.9	71.7	80.4
COVID-19 PATIENTS, CONVALESCENT PHASE (N=27)	IgG	96.3	85.2	85.2	96.3	96.3
	IgA	81.5	92.6	96.3	96.3	100.0
	IgM	55.6	74.1	88.9	81.5	88.9
	IgG/IgA	96.3	96.3	100.0	100.0	100.0
	IgG/IgM	96.3	85.2	92.6	96.3	96.3
	IgA/IgM	81.5	96.3	100.0	96.3	100.0

Next, cross-reactivity between low-pathogenic and high-pathogenic hCoVs was tested. Most negative control participants were positive for NL63 (86%) and 229E (69%), and some were positive for OC43 (45%) and HKU1 (27%). However, previous infections with low-pathogenic hCoVs caused minimal cross-reactivity with antigens of high-pathogenic coronaviruses: SARS-CoV-2, SARS, and MERS N-specific IgG antibody responses were detected in 1–3% of negative control participants. Instead, SARS-CoV-2 infections boosted the low pathogenic hCoV antibody production in some participants even when hCoV-specific antibody response was low or absent. In particular, 229E and NL63 N-specific IgG antibody levels were elevated after SARS-CoV-2 infection and out of 16 patients with paired serum samples, six (38%) had >10 unit increase between acute and convalescent phase serum samples against 229E and NL63 N.

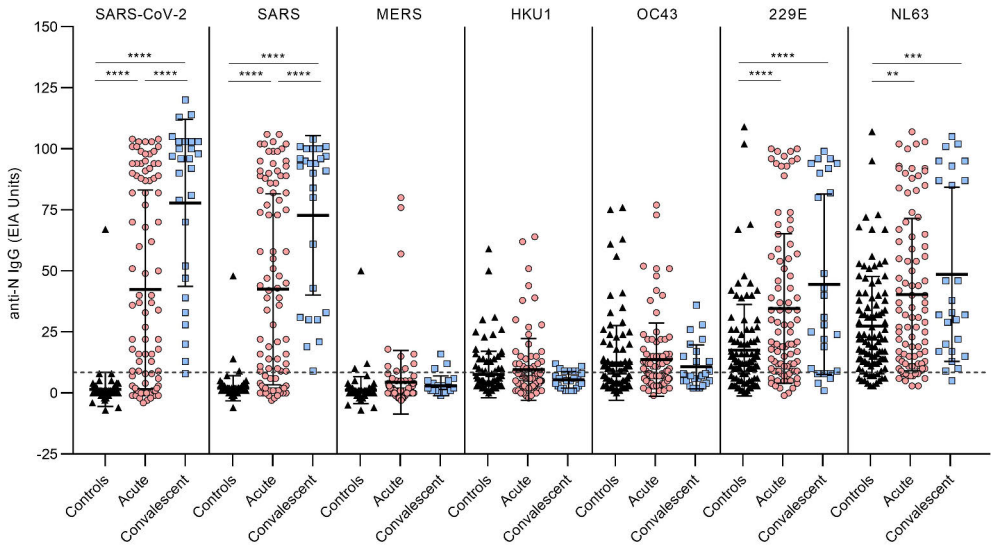

Figure 3. Antibody responses against nucleoproteins of all seven human CoVs, SARS-CoV-2, SARS, MERS, HKU1, OC43, 229E, and NL63, in serum samples collected from negative controls (n=100) and PCR-confirmed COVID-19 patients at acute (n=91) and convalescent phase (n=26). One patient with paired serum samples was excluded due to insufficient sample amount. The dotted line indicates cut-off units of 8.5, and the mean with SD is shown. Two-tailed p-values <0.05 were considered significant. Modified from Original publication I. **p<0.01, ***p<0.001, ****p<0.0001

5.2 Humoral immune response following COVID-19 mRNA vaccination (II–IV)

In studies II–IV, COVID-19 mRNA vaccine-induced immune responses were analysed in health care workers (HCWs) who received two doses of BNT162b2 (Comirnaty, Pfizer-BioNTech) vaccine as part of the occupational health care with a three-week dose interval. HCWs invited to the study cared for COVID-19 patients or handled clinical SARS-CoV-2 samples and were thus considered to be at risk of occupational exposure to SARS-CoV-2. Sequential blood samples were collected before or on the day of the first vaccine dose (Pre), three weeks after the first vaccine dose (1D3wk), and three weeks after the second vaccine dose (2D3wk). Follow-up samples were collected three and six months after the second vaccine dose (2D3mo and 2D6mo, respectively). Detailed participant characteristics, sample collection intervals, and study methods are described in the original publications.

5.2.1 Binding antibodies

COVID-19 vaccine-induced antibodies were analysed with S1 and N-based EIAs. Before vaccinations, SARS-CoV-2 infection was determined based on S1 alone or

S1 and N seropositivity due to higher similarity of SARS-CoV-2 N protein than S1 with other hCoVs. After vaccinations, the rate of SARS-CoV-2 infections was determined based on an increase in N-specific antibodies. In study II, six participants were anti-S1 IgG positive before vaccination, six were anti-N IgG positive, and five were positive for both S1 and N. The first vaccine dose induced anti-S1 IgG antibodies in 94% of initially S1-seronegative participants, and although the levels of anti-S1 IgG varied between participants from 1 to 123 units, the geometric mean (GM) was higher than in unvaccinated home-treated convalescent COVID-19 patients (47 units vs 20 units) (Figure 4a). Notably, all participants with prior infection developed high anti-S1 responses already after the first vaccine dose. The second vaccine dose boosted S1-specific IgG antibody levels in previously S1-seronegative but not in infected individuals, which resulted in comparable responses in initially seronegative and seropositive participants (GM 107 units vs 109 units, p=0.2384). S1-specific IgA and IgM levels were slightly elevated in some vaccinated individuals (Figure 4b). Of the unvaccinated COVID-19 patients, 47 (94%) had anti-S1 IgM antibodies and only four (8%) had anti-S1 IgA antibodies.

In studies III and IV, the serological follow-up was continued for a smaller subset of COVID-19 vaccinated participants without prior SARS-CoV-2 infection. Study II, III, and IV results were comparable with each other: after the second vaccine dose, GMs were 107, 109, and 125 units, respectively. After the peak antibody levels were reached, anti-S1 IgG levels started to decrease. Three months post-vaccination GMs were 88 and 98 units in studies III and IV, respectively, and the levels were higher compared to unvaccinated hospitalized COVID-19 patients (Figure 4c). Six months after the vaccination, antibody levels had decreased to levels that corresponded to 50% of the peak antibody levels. Despite the decline, all participants remained seropositive for anti-S1 antibodies, and based on anti-N antibody levels, participants did not contract SARS-CoV-2 infection during the six-month study period.

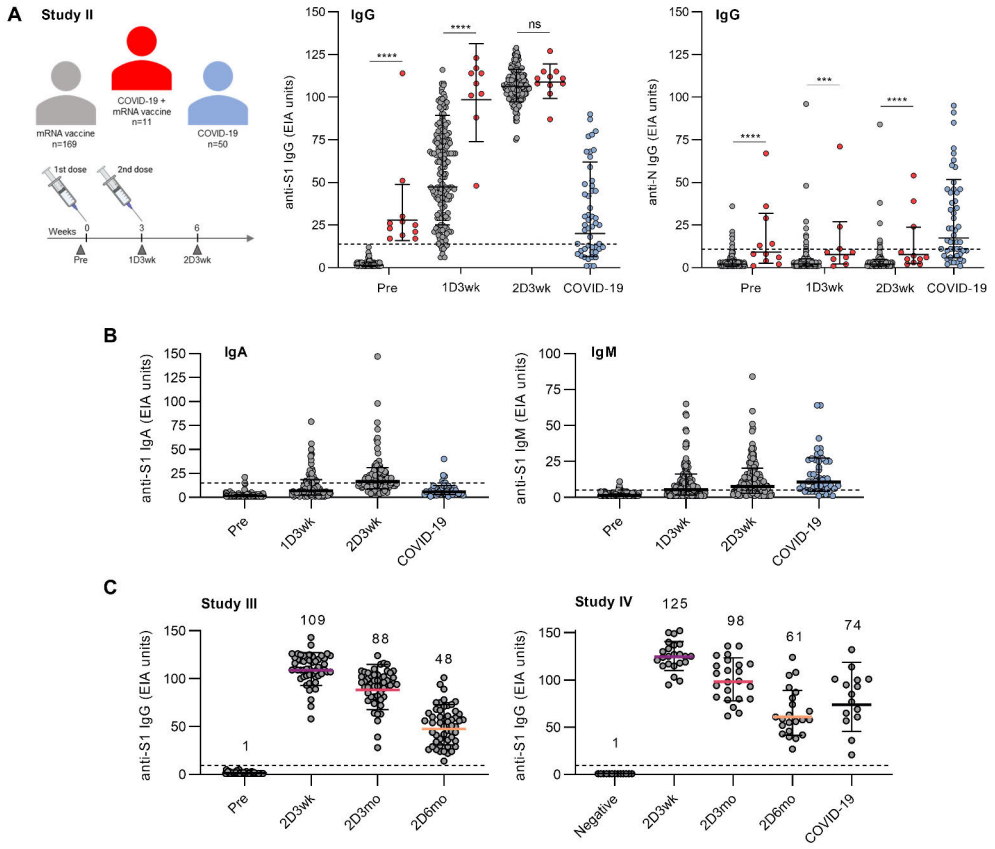

Figure 4. Antibody responses against SARS-CoV-2 S1 and N proteins in mRNA vaccinated health care workers and recovered COVID-19 patients. **A**. Anti-S1 and anti-N IgG antibody levels were measured from serum samples collected from vaccinated participants with and without previous SARS-CoV-2 infection and home-treated convalescent phase patients 2-6 weeks after positive PCR test result. **B**. Anti-S1 IgA and IgM antibodies in vaccinated and infected participants. **C**. Longevity of vaccine-induced antibody responses in a subset of vaccinated participants, COVID-19 hospitalized patients one month after symptom onset, and healthy negative controls who had not been vaccinated or infected. Geometric means are represented above the groups. Cut-off values are indicated with dashed lines. Modified from Original publications II, III, and IV. ns, not significant; ***p<0.001; ****p<0.0001

5.2.2 Neutralizing antibodies

In studies II and III, the neutralization of D614G variant (FIN-25 isolate), SR-121 isolate (Pango lineage B.1.463), and Alpha, Beta, Eta, and Delta variants was analysed with MNT from serum samples collected before and after COVID-19 vaccinations. SARS-CoV-2 naïve participants did not have cross-reactive neutralizing antibodies before vaccination, and the first vaccine dose induced neutralizing antibody response in 63% of participants against D614G (Figure 5a).

No difference was observed in the neutralization of Alpha variant compared to D614G; however, the neutralization of Beta variant was significantly reduced ($p<0.0001$, Figure 5b). In contrast, most participants with prior anti-S1 IgG antibodies showed a low neutralization activity against D614G, Alpha, and Beta variants already before the vaccination, and pre-existing immunity was found to increase the neutralization titers after the first vaccination (Figure 5a).

The second vaccine dose boosted the neutralizing antibody levels in all uninfected participants. Three weeks after the second dose, 100% of participants neutralized the D614G, SR-121, Alpha, and Eta variants. Beta variant was neutralized by 92% of the participants, while Delta variant was neutralized by 98% of the participants. The neutralization of Beta and Delta variants was significantly lower compared to D614G (Figure 5b). Although the second vaccine dose increased the neutralization titers only slightly in pre-infected participants, the neutralization titers were higher than in uninfected vaccinated participants. Furthermore, vaccinated participants neutralized variants better than non-vaccinated convalescent COVID-19 outpatients (Figure 5a). Out of the COVID-19 patients, 86%, 96%, and 56% neutralized D614G, Alpha, and Beta variants, respectively.

Similar to antibodies measured with EIA, the neutralization titers decreased three and six months after the vaccination (Figure 5c). Between three-week and six-month timepoints, the decrease in the neutralization titers was 3.9, 3.1, and 3.8-folds against D614G, Alpha, and Delta, respectively. While D614G and Alpha were neutralized by all vaccinated participants, Delta was neutralized by 85% of participants six months after the vaccinations.

In study II, to assess whether COVID-19 mRNA vaccine-induced antibody levels are associated with neutralizing antibody levels, anti-S1 IgG antibodies were compared to neutralization titres against D614G variant. Anti-S1 IgG measurement correlated highly with neutralization titres (Spearman $r=0.913$, $p<0.0001$), indicating that anti-S1 antibody measurement could be used to determine the level of protection against SARS-CoV-2 infection, at least with D614G variant.

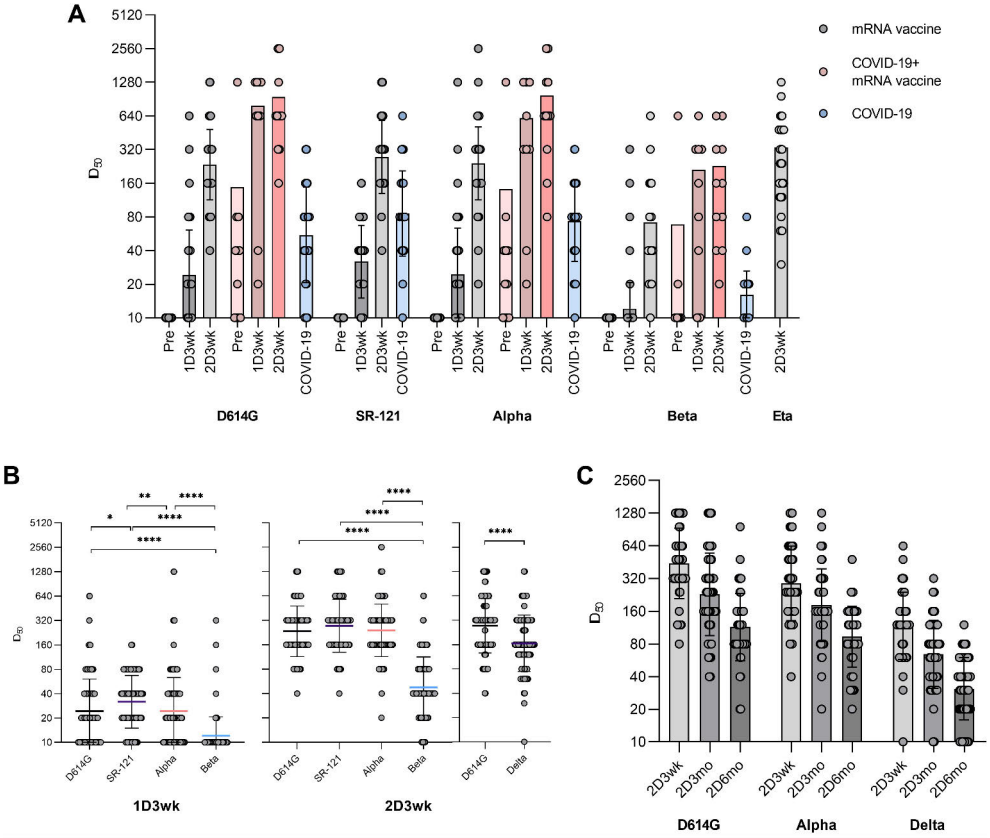

Figure 5. Neutralization of SARS-CoV-2 variants D614G, SR-121 (Pango lineage B.1.463), Alpha, Beta, Eta, and Delta in mRNA vaccinated health care workers and recovered COVID-19 patients. **A**. Neutralization by serum samples collected from vaccinated participants with and without previous SARS-CoV-2 infection and home-treated convalescent phase patients. Neutralization of Eta was measured in a separate study with similar assay settings as with other variants. **B**. Neutralization of variants was compared three weeks after the first (1D3wk) and second (2D3wk) doses. MNT for SR-121, Alpha, and Beta was performed with 3-day incubation, while MTN for Delta was done with 4-day incubation. Neutralization of the D614G variant was measured both with 3 and 4-day incubation. **C**. Neutralization of D614G, Alpha, and Delta variants three (2D3mo) and six months (2D6mo) after vaccination. Modified from Original publications II and III. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

5.2.3 Age and sex-related distribution of vaccine-induced responses

The effect of age and gender on the vaccine-induced humoral immune response was analysed in study II by dividing participants into four age groups. After the first vaccine dose, neutralization titers against D614G strain were significantly lower in the older (55–65 years) compared to younger participants (20–34 years p=0.0005 and 35–44 years p=0.0133). However, after the second vaccine dose, there was no

statistically significant difference between the age groups, although older participants had slightly lower neutralization titers compared to younger (geometric mean titer 257, 268, 200, and 206 in age groups of 20–34, 35–44, 45–54, and 55–65 years, respectively). Gender did not affect the humoral immune responses after the first vaccine dose; however, females showed slightly higher neutralization titres after the second dose than males (p=0.0412).

5.3 Whole genome sequencing of SARS-CoV-2 isolates (II–III)

Based on WGS, Alpha, Beta, Eta, and Delta isolates used in studies II and III had typical aa substitutions and deletions defining their lineages (Figure 6). Alpha carried 10 (study III) or 11 (study II), Beta 10 (study III) or 11 (study II), Eta 9, and Delta 11 aa changes in the S protein. In addition, Alpha (study III) and Eta had amino acid change R682W near the furin cleavage site (FCS) due to cell culture adaptation. SR-121 (Pango lineage B.1.463) isolate had four aa substitutions in the S protein (three in the S1 subunit; F59Y, P384L, D614G, and one in the S2 subunit; P812S), and was genetically closest to D614G compared to other isolates. D614G isolate had five deletions (study II) and one substitution (study II and III) near the FCS. Other isolates had either intact FCS or substitutions in less than a quarter of the sequences.

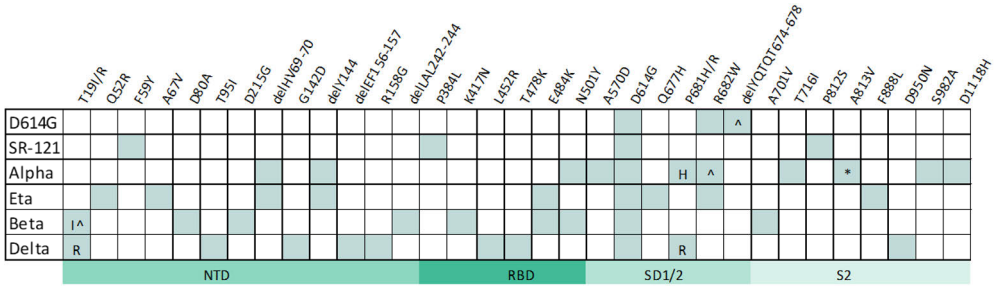

Figure 6. Amino acid changes in the spike protein based on whole genome sequencing. Changes present in over 40% of sequences are shown. Aa changes that are present only in the study II isolate are marked with ^ and aa changes only in the study III isolate are marked with *. NTD, N-terminal domain; RBD, receptor-binding domain, SD1/2, subdomains 1 and 2; S2, subunit 2

5.4 T cell responses following COVID-19 mRNA vaccination (IV)

Study IV aimed to evaluate the persistence of T cell responses six months after two COVID-19 vaccine doses. Activation of SARS-CoV-2-specific memory T cells was analysed with activation-induced marker (AIM) assay and by measuring secreted

cytokines and effector molecules (IFN-γ, IL-2, TNFα, and perforin) after stimulation of PBMCs. AIM assay was originally developed at La Jolla Institute at the University of California for the detection of pathogen-specific CD4+ T cells (Dan et al., 2016), and later adapted for SARS-CoV-2-specific T cells analysis (Grifoni et al., 2020).

5.4.1 Activation induced surface markers

After COVID-19 mRNA vaccination, S-specific memory CD4+ T cells were activated in 100%, and memory CD8+ T cells were activated in 70% of vaccinated participants (Figure 7). There was no statistically significant difference in CD4+ or CD8+ T cell activation between vaccinated individuals and hospitalized COVID-19 patients. Three and six months after the vaccination, CD4+ and CD8+ T cell responses persisted, and the mean SI-values were 8.6–9.5 for CD4+ and 1.6–2.6 for CD8+ T cells. In addition to measuring T cell activity against Wuhan-Hu-1, a number of samples were stimulated with S peptides of Alpha, Beta, Gamma and Delta variants. CD4+ T cell response was detected in 71% and CD8+ T cell response in 50% of participants against all SARS-CoV-2 variant peptide pools, indicating that T cells are able to cross-recognize genetically different strains. Some of the negative donors had also low levels of variant S-specific T cells likely due to pre-existing hCoV-specific T cells. At the six-month timepoint, a significant decrease was noted between Alpha and Beta variant-specific CD4+ T cells when paired samples were analysed; however, a consistent difference across variants was not observed.

Serum anti-S1 antibody levels in vaccinated participants did not correlate with either CD4+ or CD8+ T cell responses ($r=0.117$ and $p=0.4061$, $r=0.225$ and $p=0.1090$, respectively), further supporting the persistence of memory T cells despite decreasing antibody levels.

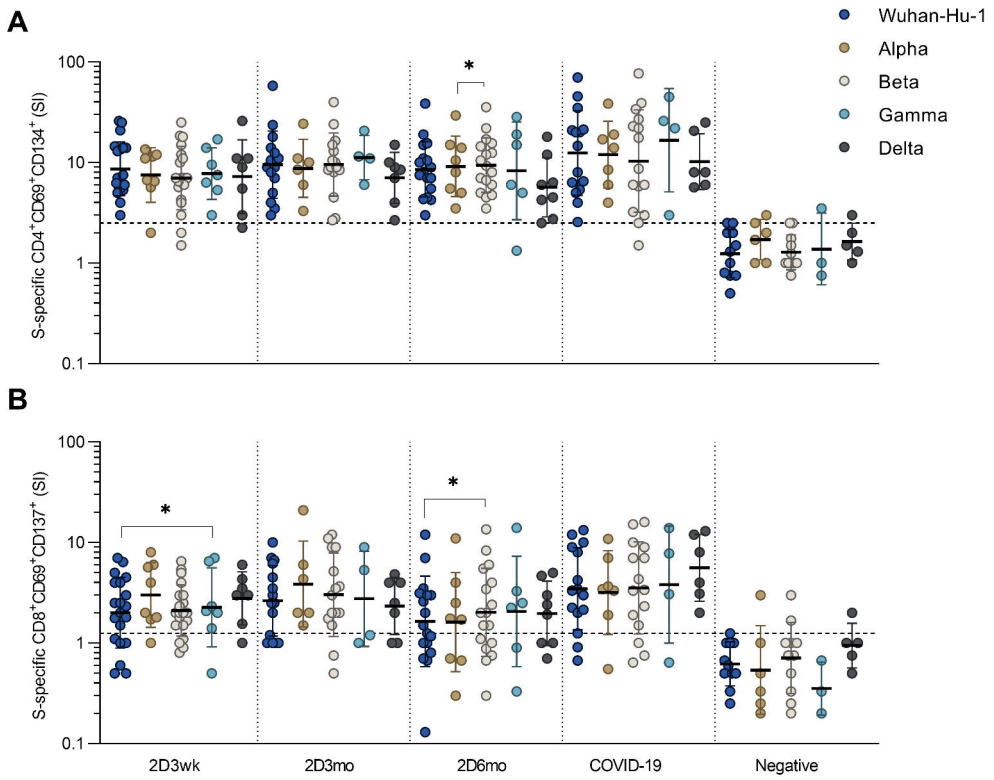

Figure 7. COVID-19 mRNA vaccine-induced T cell responses against SARS-CoV-2 original Wuhan-Hu-1 strain and variants of concern S peptide pools. **A**. S-specific CD4+ T cell responses and **B**. S-specific CD8+ T cell responses in vaccinated healthcare workers, convalescent COVID-19 patients, and negative control donors. Statistical differences were analysed between paired serum samples and samples with no data on both data points were excluded from the analysis. Modified from Original publication IV. *p<0.05

5.4.2 Secreted cytokines

PBMCs of all vaccinated participants secreted IFN-γ and IL-2 in response to stimulation with tetanus toxoid, Wuhan-Hu-1 and Delta variant S peptide pools (Figure 8). The levels of IFN-γ and IL-2 were higher in the S peptide pool stimulated vaccinated participants and COVID-19 patients than in negative controls, and the levels remained the same at all time points post-vaccination. In addition, no difference was detected between Wuhan-Hu-1 and Delta variant S peptide pool stimulated cells. Some negative participants had elevated IFN-γ and IL-2 secretion when stimulated with S peptide pools, possibly due to pre-existing cross-reactive T cells. IFN-γ concentrations correlated with CD8+ T cell activation (Spearman r=0.58, p<0.0001) and moderately with CD4+ T cell activation (r=0.47, p<0.0001), while IL-2 concentrations correlated with CD8+ T cells (r=0.55, p<0.0001) but not with CD4+ T cells (r=0.24, p=0.1090). Increased production of TNFα and perforin

was detected in most COVID-19 patients (Figure 8C–D), but only in a few vaccinated participants. Furthermore, the TNFα and perforin levels were relatively high in DMSO-stimulated PBMCs and negative control donors compared to IFN-γ and IL-2.

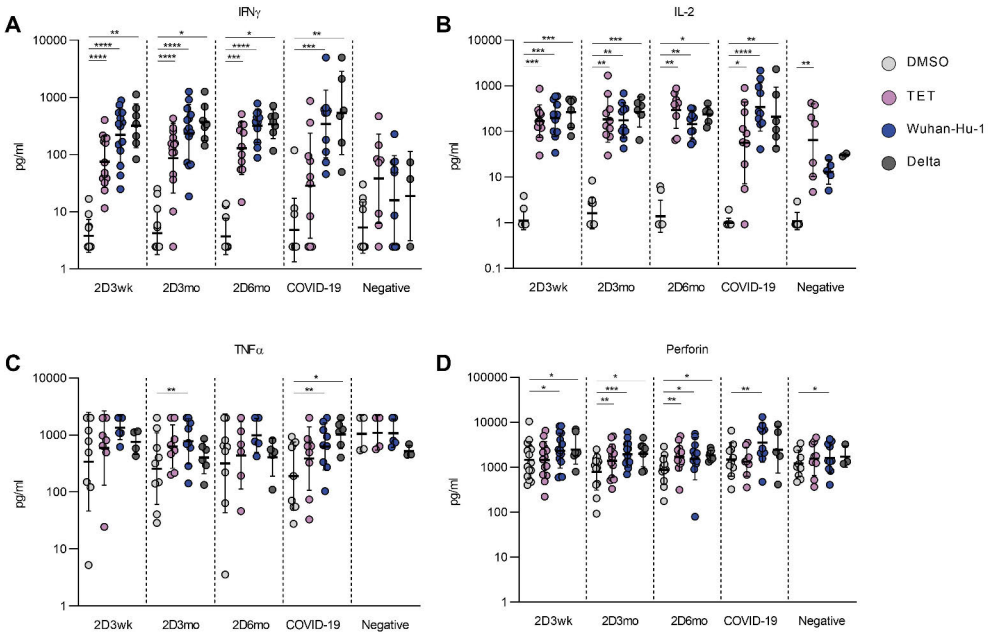

Figure 8. Secreted cytokines after stimulation of PBMCs with original Wuhan-Hu-1 virus and Delta variant spike-specific peptide pools. Stimulation with DMSO was used as negative control and stimulation with tetanus toxoid was used as positive control. The concentration of **A**. IFN-γ, **B**. IL-2, **C**. TNFα, and **D**. perforin were analysed with multiplex Luminex immunoassay. Data are represented as geometric means with geometric SD. Modified from Original publication IV. *p < 0.05; **p < 0.01; ***p < 0.001.

6 Discussion

6.1 SARS-CoV-2 serology tests

Analysis of SARS-CoV-2-specific antibodies has an essential role in estimating seroprevalence, assessing vaccine effectiveness, and analysing how SARS-CoV-2 interacts with the immune system. N and S are one of the most immunogenic proteins in SARS-CoV-2, and multiple serological assays utilizing these proteins were developed already in the spring of 2020. To date, 83 serological tests have been approved by the FDA under emergency use authorization (FDA, 2022), and 646 antibody tests have been CE-IVD marked in the European Union (European Commission, 2023). Many commercial tests have not been independently evaluated, and a wide variety of technologies and the lack of reference sample panels create challenges in applying the tests in clinical use. In addition, at the beginning of the pandemic, SARS-CoV-2 proteins were not widely available, the assays showed remarkable discrepancies, and the assay sensitivities varied between 46–100% (GeurtsvanKessel et al., 2020; Jääskeläinen et al., 2020). Therefore, we produced S1, RBD, and N proteins and developed an immunoassay for measuring IgG, IgA, and IgM antibodies against these proteins.

The fusion of S1 and RBD with mFc increased the yield in protein production and the signals in EIA. The sensitivities for anti-RBD-mFc and anti-S1-mFc IgG tests were 41% and 49%, respectively, when samples were collected during the first two weeks post symptom onset, and 85% when samples were collected ≥14 days after onset. The sensitivity of the anti-N IgG EIA was higher in the early and late stages of the disease (65% and 96%, respectively), and the specificities of all tests were 98–99%. The sensitivity and specificity of our in-house EIA resembled the performance of commercial tests, which were validated with samples collected at least two weeks after SARS-CoV-2 infection (Lagerqvist et al., 2021; Piec et al., 2021). In addition, two meta-analyses determined that the sensitivity of EIA tests increased from 65% to 82–99% when samples were collected during the second week of infection or during the third week and later (Lisboa Bastos et al., 2020; Macedo et al., 2022).

The lower sensitivity of the tests at the early stage of COVID-19 is explained by the mean seroconversion time of 12 days after symptom onset (Iyer et al., 2020; Long

et al., 2020; Lou et al., 2020). Peak anti-S IgG antibodies are reached in three weeks, and Van Elslande et al. reported that N-specific antibodies are detected before anti-S antibodies (Van Elslande et al., 2020). In addition, IgM and IgA antibodies have been shown to peak earlier than IgG in some patients (Iyer et al., 2020), and thus IgA and IgM tests can potentially detect seroconversion earlier than the IgG test. Indeed, we found that by combining IgG and IgA measurements, the assay's sensitivity could be increased both at acute and convalescent phases of the disease. Also, a combination of IgA and IgM improved the assay performance; however, both IgA and IgM antibodies have been shown to decrease rapidly after infection and return to base level in two to three months (Iyer et al., 2020; Nathalie et al., 2021). Therefore, combining N and S-specific antibody measurements with IgG and IgA/IgM could increase the accuracy of seroprevalence estimates by detecting those who contracted SARS-CoV-2 recently or a few months ago.

Antibody production in response to viral infections differs between patients and can be affected by age, sex, presence of comorbidities, and medication. Interestingly, in SARS-CoV-2 infections, antibody kinetics are affected by disease severity (Lucas et al., 2021). Recent studies have demonstrated that S and N-specific IgG and IgM responses are higher in hospitalized patients than in outpatients (Nathalie et al., 2021; Röltgen et al., 2020) and disease severity has also been associated with persistent antibody levels (Chia et al., 2021). Wei et al. showed that patients who did not develop antibodies were older, asymptomatic, or had low viral burden detected by RT-qPCR (Wei et al., 2021). We did not have any data on the disease severity, and it remains unclear whether, in our patient cohort, some seronegative patients with only acute phase samples had mild or asymptomatic infection and failed to seroconvert or seroconverted at a later stage.

Seasonal human coronavirus N-proteins share 24–36% amino acid sequence identity with SARS-CoV-2 N, and thus, determining the cross-reactivity of pre-pandemic samples with SARS-CoV-2 antigens was important. Although our pre-pandemic serum samples had anti-NL63, 229E, and OC43 N IgG antibodies, we did not detect strong cross-reactivity with the SARS-CoV-2 or SARS N protein (1–3%). The level of cross-reactivity we detected was lower than the 11% cross-reactivity shown in another study (Galipeau et al., 2021) and it is unclear whether the cross-reactive antibodies found in some individuals are protective against SARS-CoV-2 infection or severe COVID-19.

Interestingly, NL63 and 229E N-specific IgG antibodies were boosted in some patients after SARS-CoV-2 infection but not antibodies against OC43 or HKU1, which share higher homology with SARS-CoV-2. In contrast to our results, Prevost et al. and Anderson et al. observed an increase in OC43 S-specific antibody levels after COVID-19 infection and a substantially lower increase in 229E S-specific antibodies (Anderson et al., 2021; Prévost et al., 2020). Also, SARS infection has

been associated with an increase in pre-existing antibodies against OC43, HKU1, and 229E (Chan et al., 2005; Che et al., 2005). In our study, the detection of increased NL63 and 229E N-specific antibody levels could be due to the reactivation of N-specific memory B cells formed in earlier 229E and NL63 infections, which are relatively common in Finland based on the high seropositivity. Further studies would be needed to determine whether SARS-CoV-2 infection boosts hCoV S-specific antibodies, which are more prevalent against OC43 than against 229E and NL63 (Kolehmainen et al., 2023).

SARS-CoV-2 S-specific antibodies were shown to be associated with neutralizing antibodies in our study and a number of other studies (Peluso et al., 2023; Suthar et al., 2020; Terreri et al., 2022). However, since SARS-CoV-2 is constantly evolving, measuring antibody responses against Wuhan-like S-protein will unlikely accurately estimate population-level immunity. Nevertheless, the COVID-19 serology tests can be used to monitor the prevalence of infections in the selected population and the individual's immunity over time. Serology testing can also provide valuable insights into the COVID-19 pandemic when combined with other diagnostic tests and clinical information.

6.2 COVID-19 vaccine-induced adaptive immunity

All vaccines authorized by EMA in 2020-2021 target the whole S glycoprotein (EMA, 2023). In healthy immunocompetent adults, COVID-19 vaccinations are shown to induce both humoral and cell-mediated immune responses within a month after the first vaccination. CD4+ T cells are needed to generate a robust humoral response, and neutralizing antibodies can prevent the disease by restricting the entry of SARS-CoV-2 to host cells. Although antibody levels in the blood decrease over time, re-exposure through infection or vaccination can reactivate the formed memory B and T cells (Alsoussi et al., 2023; Terreri et al., 2022).

6.2.1 Antibody responses to COVID-19 vaccination

In the phase-I/II trial, the BNT162b2 COVID-19 mRNA vaccine was shown to induce high anti-S antibodies in adults, and one week after the second dose, the antibody levels were higher than in convalescent COVID-19 patients (Walsh et al., 2020). After the authorization of the vaccines, multiple independent studies confirmed the findings of the clinical trials (Goel et al., 2021; Payne et al., 2021; Sahin et al., 2021), including our study II, which was conducted during Spring 2021 and included 180 HCWs vaccinated with BNT162b2. After the first vaccine dose, the antibody responses varied between the participants. Unlike in the phase-I/II trial, nine participants remained seronegative for S protein. Out of the nine, six

seronegative participants were older (55–65y), and age-related immunosenescence likely explains some of the missing responses (D. A. Collier et al., 2021; Goronzy & Weyand, 2013). However, the second dose boosted the antibody production in all age groups, and S-specific antibody concentrations were higher in all vaccinated participants compared to convalescent non-hospitalized patients.

Previous SARS-CoV-2 infections have been shown to affect the immune response to vaccination (Prendecki et al., 2021; Z. Wang, Muecksch, et al., 2021). A large prospective cohort study confirmed that anti-S antibody concentrations were over seven times higher in previously infected than in infection-naïve participants after vaccination (Angyal et al., 2022). In addition, the antibody levels remained higher in infected participants with one vaccine dose than in naïve participants with two vaccine doses. A recent study analysed the effect of hybrid immunity (induced by both infection and vaccinations) on antibody production and revealed that infected individuals who were vaccinated had two times more memory B-cells than uninfected, and the produced antibodies had higher neutralization potency (Andreano et al., 2021). Although our sample size of previously infected was small, we also noticed that one vaccine dose was sufficient to induce high antibody levels in participants with prior infection, and the antibody levels were not substantially increased after the second vaccine dose.

In our study, some participants produced S-specific IgA and IgM antibodies after the first and second vaccine doses, although the levels were lower than those observed for IgG antibodies. Similarly, Ruggiero et al. demonstrated that after BNT162b2 vaccination, 64% produced IgM antibodies simultaneously or following IgG antibodies (Ruggiero et al., 2022). In addition, Kalimuddin et al. reported the production of S-specific IgM and IgA antibodies ten days after BNT162b2 vaccination, and all three antibody types were found in vaccinated individuals at 21 days post-first vaccination (Kalimuddin et al., 2021). However, COVID-19 vaccination does not boost IgA production in saliva, indicating a strong systemic response with no activation of mucosal immunity (Azzi et al., 2022).

Previous studies have shown a decline in N- and S-specific antibodies after SARS-CoV-2 infection; however, antibodies seem to persist for at least one year after recovery (Haveri et al., 2021; Marcotte et al., 2022). Similarly, COVID-19 mRNA vaccine studies have demonstrated that anti-S IgG antibodies decrease a few weeks after vaccination but remain at detectable levels for at least eight months (Korosec et al., 2022). In our cohort, S1-specific antibody responses declined approximately 1.5-fold (1.2–2.1) every three months after vaccination, and as in other studies (Doria-Rose et al., 2021; Goel et al., 2021), all participants had a measurable level of antibodies at six months after vaccination. We did not determine the half-life of the antibodies; however, other studies have estimated that anti-S IgG half-life is 28–52 days in two-times vaccinated individuals (Doria-Rose et al., 2021;

Goel et al., 2021; Wei et al., 2022) or 109 days if decay rates are assumed to decrease over time (Doria-Rose et al., 2021). Anti-S half-life is estimated to be longer for those who have been infected with SARS-CoV-2 before vaccination, 266 days (Wei et al., 2022). Based on one model, antibodies are lost eight months after the second dose (Korosec et al., 2022), which supports the administration of booster doses after two vaccine doses.

6.2.2 Neutralization of evolving SARS-CoV-2 variants

SARS-CoV-2-induced neutralizing antibodies bind to the S protein blocking the virus from entering the host cell and providing protection against symptomatic infection (Khoury et al., 2021). Neutralizing antibodies against SARS-CoV-2 are commonly measured with an infection inhibition test, which includes the addition of serum dilution and live virus mixture on a cell monolayer. The results are detected as cytopathic variables, such as plaque or focus reduction (Bewley et al., 2021). Other methods include pseudotyped virus and surrogate neutralization tests, which do not require BSL3 facilities but lack other SARS-CoV-2 antigens than S providing only an approximate of neutralization (Bewley et al., 2021; C. W. Tan et al., 2020). In studies II and III, we set up a live virus MNT to measure the neutralization capacity of vaccine-induced antibodies against SARS-CoV-2 variants circulating in the Finnish population during 2020–2021: Alpha, Beta, Eta, and Delta.

SARS-CoV-2 is constantly evolving, and although most mutations have no or little impact, the accumulation of changes offers increased infectivity, higher transmissibility, and evasion of infection and vaccine-induced immunity. In particular, changes in the S-protein are of great concern since most neutralizing antibodies and therapeutic monoclonal antibodies target epitopes in the S1 subunit (Graham et al., 2021). RBD mutations, including E484K, K417N, and N501Y in Beta variant and L452K and T478K in Delta variant, have been associated with decreased neutralization and increasing immune evasion properties of the virus (Wibmer et al., 2021). N501Y in Alpha, Beta, and Gamma has been shown to increase the ACE2 binding (H. Liu et al., 2021), and in Alpha and Eta variants, deletion at HV69-70 has been proposed to increase S1/S2 cleavage and S incorporation into virions (Meng et al., 2021). P681H in Alpha is thought to increase the accessibility of the FCS and increase the resistance to beta interferon (Jose et al., 2022). Furthermore, P681R in Delta has been shown to increase the S1/S2 cleavage even higher than in Alpha variant increasing the replication of the virus (Y. Liu et al., 2022).

In our study, D614G, Alpha, and Eta variants were neutralized by all vaccinated participants three months after the second vaccine dose, indicating that mutations in Alpha and Eta are insufficient to significantly reduce the vaccine-induced

neutralizing antibody functions. Beta variant was neutralized less effectively than Alpha, and three weeks after the second vaccination, 92% of HCWs neutralized Beta with 6-fold lower neutralization titers than against D614G. Neutralization of Delta variant was also decreased by 4-fold compared to D614G. These results were in line with previous studies showing an 8-fold reduction in the neutralization of Beta variant followed by a 3 to 6-fold reduced neutralization of Delta variant compared to the original Wuhan-like virus (C. Liu et al., 2021; Mlcochova et al., 2021). Furthermore, Nasreen et al. estimated 89–92% VE against Alpha infection, 87% VE against Beta infection, and 92–95% VE against Delta infection after two doses of mRNA vaccine (Nasreen et al., 2022).

During the spring of 2021 in Finland, the predominant source of infections was the Alpha variant, which exhibited a transmissibility that was 43–90% higher than the preceding variant (Davies, Abbott, et al., 2021). Despite the co-occurrence of the Beta variant with Alpha, the former demonstrated lower transmissibility (Tegally et al., 2021), thus conferring a selective advantage to the Alpha variant. By June 2021, the Delta variant had superseded the Alpha variant, owing to its enhanced transmissibility of 63–167% more than Alpha (Earnest et al., 2022). In addition, the Delta variant displayed greater immunoevasive capabilities compared to ancestral strain and Alpha variant (Mlcochova et al., 2021; Tada et al., 2022). However, even though our observations revealed a decline in neutralizing antibody levels comparable to those measured with EIA, six months post-vaccination, 85% of the participants still exhibited neutralization activity against the Delta variant. Towards the end of 2021 Delta variant was replaced by Omicron variant leading to a massive surge in infections. However, at the time of the studies, Omicron variant had not yet spread globally and therefore was unavailable for analysis.

6.2.3 T cell-mediated responses to COVID-19 vaccination

The activation of T cell-mediated immunity is essential for protection from severe COVID-19 (Grifoni et al., 2020; Rydyznski Moderbacher et al., 2020). Activated CD4+ T cells are necessary for B and CD8+ T cell activation, while activated CD8+ T cells are important for clearing virus-infected cells. One dose of the BNT162b2 vaccine was shown to activate both CD4+ and CD8+ T cells in adults (Sahin et al., 2021). Similarly, our study demonstrated that the COVID-19 mRNA vaccine generated CD4+ and CD8+ T cell responses against the SARS-CoV-2 S protein. CD8+ T cell response was not detected in each of the vaccinated participants, while CD4+ T cell response was seen in all participants. Several studies have demonstrated lower expansion of S-specific CD8+ T cells compared to CD4+ T cells in response to infection or two or three vaccine doses (Grifoni et al., 2020; Guerrera et al., 2023; Naaber et al., 2021). However, the difference in T cell numbers needs to be analysed

further since CD8+ T cells might reside predominantly in tissues and all studies used a suboptimal length of 15 aa peptides for antigen presentation to CD8+ T cells through MHC class I molecules (Rammensee et al., 1993).

CD4+ and CD8+ T cells recognize multiple epitopes in the SARS-CoV-2 S protein and other structural and non-structural proteins. The strength of the response seems to correlate with the expression level of the viral protein, and in infection, T cell response is mostly directed towards S, N, and M proteins (Tarke et al., 2021). Tarke et al. identified that CD4+ and CD8+ T cells of each COVID-19 patient recognize approximately 30-40 epitopes, which distribute mainly around N, M and S. In addition, SARS-CoV-2 mutations have been shown to affect only 12% of predicted T cell epitopes, indicating efficient cross-recognition of SARS-CoV-2 variants (Nelde et al., 2021). In line with this observation, we showed that S-specific CD4+ and CD8+ T cells cross-recognized Alpha, Beta, Gamma, and Delta variant S peptides in vaccinated and infected individuals. Other studies have reported similar results and demonstrated that vaccine-induced T cells also cross-recognize recent Omicron variants (GeurtsvanKessel et al., 2022; Goel et al., 2023; Tarke et al., 2022). However, T cell responses and recognized epitopes vary between individuals, and one study demonstrated a 50% loss of T cell activity against the Omicron variant in 20% of the individuals (Naranbhai et al., 2022). Despite the individual variation, these results suggest that SARS-CoV-2 evolution is not associated with increased evasion of T cell responses at the population level.

In addition to the expression of surface activation markers, we measured the functionality of the T cells. Stimulated PBMCs (and T cells) secreted IFN-γ and IL-2 in high levels, indicating a Th1 dominant response in infected and vaccinated individuals. A similar response has been detected by multiple other studies (Painter et al., 2021; Sekine et al., 2020), and Th1-mediated T cell response is known to be important in clearing many other viral infections, including measles, varicella, and influenza. Studies have shown that influenza vaccine-induced Th1-skewed response promotes the generation of cytotoxic CD8+ T cells and rapid clearance of influenza virus infection, while Th2-skewed response has delayed the virus clearance in influenza virus-infected mice (Moran et al., 1999). In our study, we showed that levels of S-specific CD4+ T cells, IFN-γ, and IL-2 correlate with the levels of S-specific CD8+ T cells, suggesting that CD4+ T cells are able to promote the expansion of CD8+ T cells.

Consistent with previous research (GeurtsvanKessel et al., 2022), our study provides evidence that the COVID-19 mRNA vaccine elicits robust S-specific T cell responses, which remain detectable for at least six months post-vaccination. Guerrera et al. observed a slight decrease in the activation of memory CD4+ and CD8+ T cells between samples collected two weeks and six months after vaccination (Guerrera et al., 2023). However, it is important to note that the proportion of

individuals with detectable T cell responses increased over time, indicating a potential stabilization or delayed response. Although our study did not analyse the impact of a third vaccine dose, subsequent studies have demonstrated a rapid recall of T cell responses following repeated immunizations (Koutsakos et al., 2023; Maringer et al., 2023). Remarkably, individuals who recovered from SARS infection were found to possess memory T cells that could be reactivated even 17 years after infection (Le Bert et al., 2020). Therefore, it is plausible that infections with new SARS-CoV-2 variants reactivate memory CD4+ and CD8+ T cells years after vaccination, providing protection against severe illness and mortality, despite not completely preventing infection.

6.3 Limitations of the study

Our studies had limitations. At the time of the study I, COVID-19 patient serum samples were not broadly available, and the sensitivity was estimated with only 27 convalescent-phase samples. In some patients, the date for the onset of symptoms was not available, and thus the day of the positive PCR test was used to determine acute and convalescent-phase samples. The difference between the symptom onset and the first PCR test can vary by approximately 10 days (Suthar et al., 2020), and some samples could have been miscategorized as acute phase samples based only on PCR test results. In addition, we did not know the duration and severity of the symptoms experienced. Also, the WHO international standard was not yet available, and we could not calibrate our test to reduce interlaboratory variation.

In studies II-IV, HCWs were 20 to 65 years old, and the age distribution did not represent the whole vaccinated population. We did not include elderly individuals and thus we could not fully study the effect of age-dependent immunosenescence. In study III, anti-S1 IgG antibody levels saturated the assay after the second vaccine dose with the used dilution, and fold-reduction three months after vaccination was likely underestimated.

In study IV, the sample size was relatively small, and we used cryopreserved PBMCs, a procedure that can decrease cell viability. PBMCs were stimulated with 15-mers, which could underestimate the CD8+ T cell activation since the optimal peptide size for the MHC class I binding groove is smaller, 8-10aa (Trolle et al., 2016). Cytokines were measured from the PBMC supernatants, and we cannot determine the polyfunctionality of the T cells or confirm which cell types were producing the cytokines. Also, we lacked samples to measure S-specific T cell responses before vaccination in the same individuals who provided samples after immunization. Lastly, we could not assess the adaptive immune responses induced by other COVID-19 vaccine types or breakthrough infections in these studies.

6.4 Future aspects of COVID-19 vaccines

By May 2023, EMA had granted authorization to seven COVID-19 vaccines targeting the S glycoprotein, along with an inactivated whole virus vaccine. In addition to the S protein, other viral proteins are also considered as vaccine candidates. N and M proteins are attractive vaccine targets since they are more conserved than S protein and have been shown to induce T cell response (Tarke et al., 2021). Recently, a novel mRNA vaccine, BNT162b4, encoding for N, M, and ORF1ab proteins, has been shown to induce cell-mediated protective immunity in hamsters and has progressed into clinical trials (Arieta et al., 2023). Updating mRNA vaccine antigens in the form of bivalent vaccines to match the currently circulating Omicron strains has not been able to significantly increase the neutralization of Omicron strains compared to previous monovalent vaccines, likely due to immune imprinting (A. Y. Collier et al., 2023). However, there is evidence suggesting that incorporating a higher quantity of mRNA specifically targeting the Omicron strains could enhance neutralizing antibody responses and alleviate the immune imprinting (Offit, 2023).

While mRNA vaccines have demonstrated high efficacy and effectiveness in preventing severe disease, administering booster doses is likely not able to offer greater protection against infection or transmission of the virus compared to previous doses. Advancements in alternative vaccine technologies, such as intranasal or oral vaccines, have the potential to yield more accessible and convenient COVID-19 vaccine formulations. These alternative delivery methods could simplify vaccine administration, improve vaccine coverage, and elicit mucosal immunity at the initial site of SARS-CoV-2 entry (Singh et al., 2023). Mucosal immunity could prevent even milder disease forms and SARS-CoV-2 transmission as shown in animal studies (Alu et al., 2022); however, further investigation is necessary to establish the effectiveness and safety of intranasal vaccines in humans.

Ongoing research is needed to assess the longevity of the COVID-19 vaccine-induced immune responses and protection beyond the six months addressed in this work. A meta-analysis revealed a decline of 10 percentage points in vaccine effectiveness against severe COVID-19 disease over a six-month period (Feikin et al., 2022), while another study reported that COVID-19 mRNA vaccines remained 56% effective against COVID-19-associated mechanical ventilation or death one to two years after the last dose (DeCuir et al., 2023). Live-attenuated vaccines and the utilization of adjuvants could potentially enhance long-term immunity by intensifying innate immune responses, thereby enhancing B and T cell activation (Pulendran & Ahmed, 2011). However, the underlying reasons why some vaccines, such as those for tetanus and diphtheria, confer lifelong protection, while others, like the influenza vaccine, require yearly administration, are not fully understood (Zhian Chen et al., 2022).

For now, research should focus on the development of safe vaccines able to prevent infection. Vaccine responses in certain risk groups and the elderly should be monitored to improve the protection against severe disease and hospitalization. Lastly, ongoing endeavours should prioritize improving vaccine distribution and ensuring equitable access to vaccines for all populations, regardless of geographic location or socioeconomic status.

7 Conclusions

The main conclusions of the study were:

1. Pre-pandemic serum samples have no or little crossreactivity to SARS-CoV-2 N and S proteins, although seasonal coronavirus NL63, 229E, and OC43 infections are common in the study population.
2. COVID-19 mRNA vaccine induces SARS-CoV-2 S-specific IgG antibodies in working age adults. A slightly attenuated antibody response is observed among older individuals after the first vaccine dose. However, upon completion of the two-dose regimen, S-specific antibodies are produced uniformly among all participants.
3. SARS-CoV-2 S-specific memory CD4+ and CD8+ T cells are present in the majority of vaccinated individuals and recovered COVID-19 patients, and the Th1 cytokine profile dominates the CD4+ T cell response.
4. Vaccine-induced antibodies cross-neutralize SARS-CoV-2 variants, although neutralization of Beta and Delta variants is slightly decreased compared to ancestral strains. SARS-CoV-2 infection followed by vaccination increases the cross-neutralization of SARS-CoV-2 variants compared to only vaccination. However, accumulating genetic changes in emerging variants may increase the evasion from vaccine and infection-induced antibodies.
5. Vaccine-induced T cells demonstrate cross-recognition of SARS-CoV-2 variants, indicating that even though these variants can evade neutralizing antibodies, the vaccine maintains its effectiveness in protecting against severe illness caused by newly emerging variants.
6. S-specific antibody levels decrease within months after the vaccination, while T cell responses remain stable for at least six months. However, further research on memory B and T cell longevity is needed.
7. Vaccine and infection-induced adaptive immunity and its interactions with emerging variants needs to be studied in order to optimize vaccine strategies and control the COVID-19 pandemic.

Acknowledgements

This doctoral thesis was carried out at the Institute of Biomedicine, Faculty of Medicine, University of Turku in Turku Doctoral Programme of Molecular Medicine (TuDMM). Financial support from Turku University Foundation, Finnish Cultural Foundation, Finnish Foundation for Research on Viral Diseases, and Emil Aaltonen Foundation is gratefully acknowledged.

I would like to express my gratitude to my excellent supervisors Professor Ilkka Julkunen and Docent Laura Kakkola for giving me the opportunity to carry out this thesis work in group Julkunen. Ilkka, your guidance, endless ideas, and enthusiasm towards science has motivated me and sparked my interest to viruses and infectious diseases. Laura, your support, mentorship, and organizational skills, whether it be projects or pizza nights, have inspired me and been essential for my journey.

I wish to thank my follow-up committee members Docent Maija Vihinen-Ranta and Docent Jukka Alinikula for their support. A warm thank you for the reviewers of this work, Docent Tuija Leino and Docent Tomas Strandin for their constructive comments and valuable ideas that improved this thesis. Many thanks to Docent Maria Söderlund-Venermo for accepting the invitation to be the opponent of this thesis.

All the co-authors and collaborators of this thesis are thanked for their contribution. I especially want to thank Johanna Lempainen, Anu Kantele, and Olli Ritvos for their guidance and initiation of the projects. I also wish to acknowledge Anne Suominen, Anne-Mari Pieniniemi, Simo Miettinen, and Kaisu Kaistinen for their contribution in the sample collection and Soili Jussila for incubators full of cells. None of this would have been possible without you.

Thank you, past and present members of group Julkunen; Sari Maljanen, Hira Khan, Rickard Lundberg, Anna Kazakova, Felix He, Arttu Reinholm, Milja Belik, Antti Hurme, Jemna Heroum, Lav Tripathi, Pekka Kolehmainen, Moona Huttunen, and Eda Altan. Sari, Hira, Rickard, Anna, Felix, Arttu, Milja, and Jemna, your peer support and the exciting working atmosphere you have created have been invaluable. Antti, thank you for sharing projects and your expertise with me. Lav, Pekka, Moona, and Eda, thank you for your encouraging, kind, and wise words during this thesis process. I have had the privilege to share biosafety cabinet with most of you and I

hope you have enjoyed it as much as I have. My sincere gratitude to Sisko Tauriainen for support and shared conference trips, and to Matti Waris for always being available for discussion.

A special thank you goes to all coworkers at the 7th floor of Medisiina D. Thank you for coffee breaks, organizing hockey nights, pre-Christmas parties, and trips to Kurjenrahka. You have created positive and warm environment in our floor.

To my friends, your unwavering support and the good times we have shared have been my source of strength and joy. My family, your continuous support and genuine interest in my work have meant the world to me. Jutta, thank you for being the best twin sister and for sharing this difficult journey with me as my greatest ally. Finally, Henri, thank you for standing by me through the years, keeping me nourished, and providing a home where I'm always welcomed.

October 2023
Pinja Jalkanen

References

Airenne, K. J., Laitinen, O. H., Mähönen, A. J., & Ylä-Herttuala, S. (2009). Preparation of recombinant baculoviruses with the BVboost system. *Cold Spring Harbor Protocols*, *4*(3), 1–7. https://doi.org/10.1101/pdb.prot5181

Akkaya, M., Kwak, K., & Pierce, S. K. (2020). B cell memory: building two walls of protection against pathogens. *Nature Reviews Immunology*, *20*(4), 229–238. https://doi.org/10.1038/s41577-019-0244-2

Alimohamadi, Y., Sepandi, M., Taghdir, M., & Hosamirudsari, H. (2020). Determine the most common clinical symptoms in COVID-19 patients: a systematic review and meta-analysis. *Journal of Preventive Medicine and Hygiene*, *61*(3), E304–E312. https://doi.org/10.15167/2421-4248/jpmh2020.61.3.1530

Alsoussi, W. B., Malladi, S. K., Zhou, J. Q., Liu, Z., Ying, B., Kim, W., Schmitz, A. J., Lei, T., Horvath, S. C., Sturtz, A. J., McIntire, K. M., Evavold, B., Han, F., Scheaffer, S. M., Fox, I. F., Mirza, S. F., Parra-Rodriguez, L., Nachbagauer, R., Nestorova, B., … Ellebedy, A. H. (2023). SARS-CoV-2 Omicron boosting induces de novo B cell response in humans. *Nature*, *617*(7961), 592–598. https://doi.org/10.1038/s41586-023-06025-4

Alu, A., Chen, L., Lei, H., Wei, Y., Tian, X., & Wei, X. (2022). Intranasal COVID-19 vaccines: From bench to bed. *EBioMedicine*, *76*. https://doi.org/10.1016/j.ebiom.2022.103841

Amicone, M., Borges, V., Alves, M. J., Isidro, J., Zé-Zé, L., Duarte, S., Vieira, L., Guiomar, R., Gomes, J. P., & Gordo, I. (2022). Mutation rate of SARS-CoV-2 and emergence of mutators during experimental evolution. *Evolution, Medicine, and Public Health*, *10*(1), 142–155. https://doi.org/10.1093/emph/eoac010

Anderson, E. M., Goodwin, E. C., Verma, A., Arevalo, C. P., Bolton, M. J., Weirick, M. E., Gouma, S., McAllister, C. M., Christensen, S. R., Weaver, J., Hicks, P., Manzoni, T. B., Oniyide, O., Ramage, H., Mathew, D., Baxter, A. E., Oldridge, D. A., Greenplate, A. R., Wu, J. E., … Hensley, S. E. (2021). Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. *Cell*, *184*(7), 1858-1864.e10. https://doi.org/10.1016/j.cell.2021.02.010

Andreano, E., Paciello, I., Piccini, G., Manganaro, N., Pileri, P., Hyseni, I., Leonardi, M., Pantano, E., Abbiento, V., Benincasa, L., Giglioli, G., De Santi, C., Fabbiani, M., Rancan, I., Tumbarello, M., Montagnani, F., Sala, C., Montomoli, E., & Rappuoli, R. (2021). Hybrid immunity improves B cells and antibodies against SARS-CoV-2 variants. *Nature*, *600*(7889), 530–535. https://doi.org/10.1038/s41586-021-04117-7

Angyal, A., Longet, S., Moore, S. C., Payne, R. P., Harding, A., Tipton, T., Rongkard, P., Ali, M., Hering, L. M., Meardon, N., Austin, J., Brown, R., Skelly, D., Gillson, N., Dobson, S. L., Cross, A., Sandhar, G., Kilby, J. A., Tyerman, J. K., … Chalk, J. (2022). T-cell and antibody responses to first BNT162b2 vaccine dose in previously infected and SARS-CoV-2-naive UK health-care workers: a multicentre prospective cohort study. *The Lancet Microbe*, *3*(1), e21–e31. https://doi.org/10.1016/S2666-5247(21)00275-5

Arieta, C. M., Xie, Y. J., Rothenberg, D. A., Diao, H., Harjanto, D., Meda, S., Marquart, K., Koenitzer, B., Sciuto, T. E., Lobo, A., Zuiani, A., Krumm, S. A., Cadima Couto, C. I., Hein, S., Heinen, A.

P., Ziegenhals, T., Liu-Lupo, Y., Vogel, A. B., Srouji, J. R., … Poran, A. (2023). The T-cell-directed vaccine BNT162b4 encoding conserved non-spike antigens protects animals from severe SARS-CoV-2 infection. *Cell*, *186*(11), 2392-2409.e21. https://doi.org/10.1016/j.cell.2023.04.007

Armando, F., Beythien, G., Kaiser, F. K., Allnoch, L., Heydemann, L., Rosiak, M., Becker, S., Gonzalez-Hernandez, M., Lamers, M. M., Haagmans, B. L., Guilfoyle, K., van Amerongen, G., Ciurkiewicz, M., Osterhaus, A. D. M. E., & Baumgärtner, W. (2022). SARS-CoV-2 Omicron variant causes mild pathology in the upper and lower respiratory tract of hamsters. *Nature Communications*, *13*(1), 3519. https://doi.org/10.1038/s41467-022-31200-y

Asahi-Ozaki, Y., Yoshikawa, T., Iwakura, Y., Suzuki, Y., Tamura, S., Kurata, T., & Sata, T. (2004). Secretory IgA antibodies provide cross-protection against infection with different strains of influenza B virus. *Journal of Medical Virology*, *74*(2), 328–335. https://doi.org/https://doi.org/10.1002/jmv.20173

Augustin, M., Schommers, P., Stecher, M., Dewald, F., Gieselmann, L., Gruell, H., Horn, C., Vanshylla, K., Cristanziano, V. Di, Osebold, L., Roventa, M., Riaz, T., Tschernoster, N., Altmueller, J., Rose, L., Salomon, S., Priesner, V., Luers, J. C., Albus, C., … Lehmann, C. (2021). Post-COVID syndrome in non-hospitalised patients with COVID-19: a longitudinal prospective cohort study. *The Lancet Regional Health – Europe*, *6*. https://doi.org/10.1016/j.lanepe.2021.100122

Ayoubkhani, D., Bosworth, M. L., King, S., Pouwels, K. B., Glickman, M., Nafilyan, V., Zaccardi, F., Khunti, K., Alwan, N. A., & Walker, A. S. (2022). Risk of Long COVID in People Infected With Severe Acute Respiratory Syndrome Coronavirus 2 After 2 Doses of a Coronavirus Disease 2019 Vaccine: Community-Based, Matched Cohort Study. *Open Forum Infectious Diseases*, *9*(9), ofac464. https://doi.org/10.1093/ofid/ofac464

Azzi, L., Dalla Gasperina, D., Veronesi, G., Shallak, M., Ietto, G., Iovino, D., Baj, A., Gianfagna, F., Maurino, V., Focosi, D., Maggi, F., Ferrario, M. M., Dentali, F., Carcano, G., Tagliabue, A., Maffioli, L. S., Accolla, R. S., & Forlani, G. (2022). Mucosal immune response in BNT162b2 COVID-19 vaccine recipients. *EBioMedicine*, *75*. https://doi.org/10.1016/j.ebiom.2021.103788

Bange, E. M., Han, N. A., Wileyto, P., Kim, J. Y., Gouma, S., Robinson, J., Greenplate, A. R., Hwee, M. A., Porterfield, F., Owoyemi, O., Naik, K., Zheng, C., Galantino, M., Weisman, A. R., Ittner, C. A. G., Kugler, E. M., Baxter, A. E., Oniyide, O., Agyekum, R. S., … Huang, A. C. (2021). CD8+ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nature Medicine*, *27*(7), 1280–1289. https://doi.org/10.1038/s41591-021-01386-7

Barrs, V., Peiris, M., Tam, K. W. S., Law, P. Y. T., Brackman, C., To, E. M. W., Yu, V. Y. T., Chu, D. K. W., Perera, R. A. P. M., & Sit, T. H. C. (2020). SARS-CoV-2 in Quarantined Domestic Cats from COVID-19 Households or Close Contacts, Hong Kong, China. *Emerging Infectious Disease Journal*, *26*(12), 3071. https://doi.org/10.3201/eid2612.202786

Bewley, K. R., Coombes, N. S., Gagnon, L., McInroy, L., Baker, N., Shaik, I., St-Jean, J. R., St-Amant, N., Buttigieg, K. R., Humphries, H. E., Godwin, K. J., Brunt, E., Allen, L., Leung, S., Brown, P. J., Penn, E. J., Thomas, K., Kulnis, G., Hallis, B., … Charlton, S. (2021). Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. *Nature Protocols*, *16*(6), 3114–3140. https://doi.org/10.1038/s41596-021-00536-y

Bidokhti, M. R. M., Tråvén, M., Krishna, N. K., Munir, M., Belák, S., Alenius, S., & Cortey, M. (2013). Evolutionary dynamics of bovine coronaviruses: natural selection pattern of the spike gene implies adaptive evolution of the strains. *Journal of General Virology*, *94*(9), 2036–2049. https://doi.org/https://doi.org/10.1099/vir.0.054940-0

Bobrovitz, N., Ware, H., Ma, X., Li, Z., Hosseini, R., Cao, C., Selemon, A., Whelan, M., Premji, Z., Issa, H., Cheng, B., Abu Raddad, L. J., Buckeridge, D. L., Van Kerkhove, M. D., Piechotta, V., Higdon, M. M., Wilder-Smith, A., Bergeri, I., Feikin, D. R., … Subissi, L. (2023). Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant

and severe disease: a systematic review and meta-regression. *The Lancet Infectious Diseases*, *23*(5), 556–567. https://doi.org/10.1016/S1473-3099(22)00801-5

Boni, M. F., Lemey, P., Jiang, X., Lam, T. T.-Y., Perry, B. W., Castoe, T. A., Rambaut, A., & Robertson, D. L. (2020). Evolutionary origins of the SARS-CoV-2 sarbecovirus lineage responsible for the COVID-19 pandemic. *Nature Microbiology*, *5*(11), 1408–1417. https://doi.org/10.1038/s41564-020-0771-4

Brouwer, P. J. M., Caniels, T. G., van der Straten, K., Snitselaar, J. L., Aldon, Y., Bangaru, S., Torres, J. L., Okba, N. M. A., Claireaux, M., Kerster, G., Bentlage, A. E. H., van Haaren, M. M., Guerra, D., Burger, J. A., Schermer, E. E., Verheul, K. D., van der Velde, N., van der Kooi, A., van Schooten, J., … van Gils, M. J. (2020). Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science*, *369*(6504), 643–650. https://doi.org/10.1126/science.abc5902

Burdin, N., Handy, L. K., & Plotkin, S. A. (2017). What Is Wrong with Pertussis Vaccine Immunity? The Problem of Waning Effectiveness of Pertussis Vaccines. *Cold Spring Harbor Perspectives in Biology*, *9*(12). https://doi.org/10.1101/cshperspect.a029454

Cao, Y., Jian, F., Wang, J., Yu, Y., Song, W., Yisimayi, A., Wang, J., An, R., Chen, X., Zhang, N., Wang, Y., Wang, P., Zhao, L., Sun, H., Yu, L., Yang, S., Niu, X., Xiao, T., Gu, Q., … Xie, X. S. (2023). Imprinted SARS-CoV-2 humoral immunity induces convergent Omicron RBD evolution. *Nature*, *614*(7948), 521–529. https://doi.org/10.1038/s41586-022-05644-7

CDC. (2021). *Estimated COVID-19 Burden*.

Cervia, C., Nilsson, J., Zurbuchen, Y., Valaperti, A., Schreiner, J., Wolfensberger, A., Raeber, M. E., Adamo, S., Weigang, S., Emmenegger, M., Hasler, S., Bosshard, P. P., De Cecco, E., Bächli, E., Rudiger, A., Stüssi-Helbling, M., Huber, L. C., Zinkernagel, A. S., Schaer, D. J., … Boyman, O. (2021). Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. *Journal of Allergy and Clinical Immunology*, *147*(2), 545-557.e9. https://doi.org/10.1016/j.jaci.2020.10.040

Chan-Yeung, M., & Xu, R.-H. (2003). SARS: epidemiology. *Respirology*, *8*(s1), S9–S14. https://doi.org/https://doi.org/10.1046/j.1440-1843.2003.00518.x

Chan, K. H., Cheng, V. C. C., Woo, P. C. Y., Lau, S. K. P., Poon, L. L. M., Guan, Y., Seto, W. H., Yuen, K. Y., & Peiris, J. S. M. (2005). Serological Responses in Patients with Severe Acute Respiratory Syndrome Coronavirus Infection and Cross-Reactivity with Human Coronaviruses 229E, OC43, and NL63. *Clinical and Vaccine Immunology*, *12*(11), 1317–1321. https://doi.org/10.1128/CDLI.12.11.1317-1321.2005

Chandrashekar, A., Liu, J., Martinot, A. J., McMahan, K., Mercado, N. B., Peter, L., Tostanoski, L. H., Yu, J., Maliga, Z., Nekorchuk, M., Busman-Sahay, K., Terry, M., Wrijil, L. M., Ducat, S., Martinez, D. R., Atyeo, C., Fischinger, S., Burke, J. S., Slein, M. D., … Barouch, D. H. (2020). SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science*, *369*(6505), 812–817. https://doi.org/10.1126/science.abc4776

Chaw, S.-M., Tai, J.-H., Chen, S.-L., Hsieh, C.-H., Chang, S.-Y., Yeh, S.-H., Yang, W.-S., Chen, P.-J., & Wang, H.-Y. (2020). The origin and underlying driving forces of the SARS-CoV-2 outbreak. *Journal of Biomedical Science*, *27*(1), 73. https://doi.org/10.1186/s12929-020-00665-8

Che, X. Y., Qiu, L. W., Liao, Z. Y., Wang, Y. Di, Wen, K., Pan, Y. X., Hao, W., Mei, Y. B., Cheng, V. C. C., & Yuen, K. Y. (2005). Antigenic cross-reactivity between severe acute respiratory syndrome-associated coronavirus and human coronaviruses 229E and OC43. *Journal of Infectious Diseases*, *191*(12), 2033–2037. https://doi.org/10.1086/430355

Chen, Jiahui, Wang, R., Gilby, N. B., & Wei, G.-W. (2022). Omicron Variant (B.1.1.529): Infectivity, Vaccine Breakthrough, and Antibody Resistance. *Journal of Chemical Information and Modeling*, *62*(2), 412–422. https://doi.org/10.1021/acs.jcim.1c01451

Chen, Jidang, Wang, J., Zhang, J., & Ly, H. (2021). Advances in Development and Application of Influenza Vaccines. *Frontiers in Immunology*, *Jul 13*(12). https://doi.org/10.3389/fimmu.2021.711997

Chen, X., Pan, Z., Yue, S., Yu, F., Zhang, J., Yang, Y., Li, R., Liu, B., Yang, X., Gao, L., Li, Z., Lin, Y., Huang, Q., Xu, L., Tang, J., Hu, L., Zhao, J., Liu, P., Zhang, G., … Ye, L. (2020). Disease severity dictates SARS-CoV-2-specific neutralizing antibody responses in COVID-19. *Signal Transduction and Targeted Therapy*, *5*(1), 180. https://doi.org/10.1038/s41392-020-00301-9

Chen, Zeyu, & John Wherry, E. (2020). T cell responses in patients with COVID-19. *Nature Reviews Immunology*, *20*(9), 529–536. https://doi.org/10.1038/s41577-020-0402-6

Chen, Zhian, Gao, X., & Yu, D. (2022). Longevity of vaccine protection: Immunological mechanism, assessment methods, and improving strategy. *VIEW*, *3*(1), 20200103. https://doi.org/https://doi.org/10.1002/VIW.20200103

Chia, W. N., Zhu, F., Ong, S. W. X., Young, B. E., Fong, S.-W., Le Bert, N., Tan, C. W., Tiu, C., Zhang, J., Tan, S. Y., Pada, S., Chan, Y.-H., Tham, C. Y. L., Kunasegaran, K., Chen, M. I.-C., Low, J. G. H., Leo, Y.-S., Renia, L., Bertoletti, A., … Wang, L.-F. (2021). Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *The Lancet Microbe*, *2*(6), e240–e249. https://doi.org/10.1016/S2666-5247(21)00025-2

Cohen, C., Kleynhans, J., von Gottberg, A., McMorrow, M. L., Wolter, N., Bhiman, J. N., Moyes, J., du Plessis, M., Carrim, M., Buys, A., Martinson, N. A., Kahn, K., Tollman, S., Lebina, L., Wafawanaka, F., du Toit, J. D., Gómez-Olivé, F. X., Dawood, F. S., Mkhencele, T., … Tempia, S. (2022). SARS-CoV-2 incidence, transmission, and reinfection in a rural and an urban setting: results of the PHIRST-C cohort study, South Africa, 2020-21. *The Lancet Infectious Diseases*, *22*(6), 821–834. https://doi.org/10.1016/S1473-3099(22)00069-X

Cohen, K. W., Linderman, S. L., Moodie, Z., Czartoski, J., Lai, L., Mantus, G., Norwood, C., Nyhoff, L. E., Edara, V. V., Floyd, K., De Rosa, S. C., Ahmed, H., Whaley, R., Patel, S. N., Prigmore, B., Lemos, M. P., Davis, C. W., Furth, S., O'Keefe, J. B., … McElrath, M. J. (2021). Longitudinal analysis shows durable and broad immune memory after SARS-CoV-2 infection with persisting antibody responses and memory B and T cells. *Cell Reports Medicine*, *2*(7), 100354. https://doi.org/https://doi.org/10.1016/j.xcrm.2021.100354

Collier, A. Y., Miller, J., Hachmann, N. P., McMahan, K., Liu, J., Bondzie, E. A., Gallup, L., Rowe, M., Schonberg, E., Thai, S., Barrett, J., Borducchi, E. N., Bouffard, E., Jacob-Dolan, C., Mazurek, C. R., Mutoni, A., Powers, O., Sciacca, M., Surve, N., … Barouch, D. H. (2023). Immunogenicity of BA.5 Bivalent mRNA Vaccine Boosters. *New England Journal of Medicine*, *388*(6), 565–567. https://doi.org/10.1056/NEJMc2213948

Collier, D. A., Ferreira, I. A. T. M., Kotagiri, P., Datir, R. P., Lim, E. Y., Touizer, E., Meng, B., Abdullahi, A., Baker, S., Dougan, G., Hess, C., Kingston, N., Lehner, P. J., Lyons, P. A., Matheson, N. J., Owehand, W. H., Saunders, C., Summers, C., Thaventhiran, J. E. D., … Gupta, R. K. (2021). Age-related immune response heterogeneity to SARS-CoV-2 vaccine BNT162b2. *Nature*, *596*(7872), 417–422. https://doi.org/10.1038/s41586-021-03739-1

Corripio-Miyar, Y., Hayward, A., Lemon, H., Sweeny, A. R., Bal, X., Kenyon, F., Pilkington, J. G., Pemberton, J. M., Nussey, D. H., & McNeilly, T. N. (2022). Functionally distinct T-helper cell phenotypes predict resistance to different types of parasites in a wild mammal. *Scientific Reports*, *12*(1), 3197. https://doi.org/10.1038/s41598-022-07149-9

Coutinho, R. M., Marquitti, F. M. D., Ferreira, L. S., Borges, M. E., da Silva, R. L. P., Canton, O., Portella, T. P., Poloni, S., Franco, C., Plucinski, M. M., Lessa, F. C., da Silva, A. A. M., Kraenkel, R. A., de Sousa Mascena Veras, M. A., & Prado, P. I. (2021). Model-based estimation of transmissibility and reinfection of SARS-CoV-2 P.1 variant. *Communications Medicine*, *1*(1), 48. https://doi.org/10.1038/s43856-021-00048-6

Crossley, B. M., Mock, R. E., Callison, S. A., & Hietala, S. K. (2012). Identification and characterization of a novel alpaca respiratory coronavirus most closely related to the human coronavirus 229E. *Viruses*, *4*(12), 3689–3700. https://doi.org/10.3390/v4123689

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

Dai, S., Wang, H., & Deng, F. (2018). Advances and challenges in enveloped virus-like particle (VLP)-based vaccines. *Journal of Immunological Sciences*, *2*(2), 36–41. https://doi.org/10.29245/2578-3009/2018/2.1118

Dan, J. M., Lindestam Arlehamn, C. S., Weiskopf, D., da Silva Antunes, R., Havenar-Daughton, C., Reiss, S. M., Brigger, M., Bothwell, M., Sette, A., & Crotty, S. (2016). A Cytokine-Independent Approach To Identify Antigen-Specific Human Germinal Center T Follicular Helper Cells and Rare Antigen-Specific CD4+ T Cells in Blood. *The Journal of Immunology*, *197*(3), 983–993. https://doi.org/10.4049/jimmunol.1600318

Davies, N. G., Abbott, S., Barnard, R. C., Jarvis, C. I., Kucharski, A. J., Munday, J. D., Pearson, C. A. B., Russell, T. W., Tully, D. C., Washburne, A. D., Wenseleers, T., Gimma, A., Waites, W., Wong, K. L. M., van Zandvoort, K., Silverman, J. D., Diaz-Ordaz, K., Keogh, R., Eggo, R. M., … Edmunds, W. J. (2021). Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science*, *372*(6538), eabg3055. https://doi.org/10.1126/science.abg3055

Davies, N. G., Jarvis, C. I., van Zandvoort, K., Clifford, S., Sun, F. Y., Funk, S., Medley, G., Jafari, Y., Meakin, S. R., Lowe, R., Quaife, M., Waterlow, N. R., Eggo, R. M., Lei, J., Koltai, M., Krauer, F., Tully, D. C., Munday, J. D., Showering, A., … Keogh, R. H. (2021). Increased mortality in community-tested cases of SARS-CoV-2 lineage B.1.1.7. *Nature*, *593*(7858), 270–274. https://doi.org/10.1038/s41586-021-03426-1

Davis, H. E., Assaf, G. S., McCorkell, L., Wei, H., Low, R. J., Re'em, Y., Redfield, S., Austin, J. P., & Akrami, A. (2021). Characterizing long COVID in an international cohort: 7 months of symptoms and their impact. *EClinicalMedicine*, *38*, 101019. https://doi.org/https://doi.org/10.1016/j.eclinm.2021.101019

Davis, H. E., McCorkell, L., Vogel, J. M., & Topol, E. J. (2023). Long COVID: major findings, mechanisms and recommendations. *Nature Reviews Microbiology*, *21*(3), 133–146. https://doi.org/10.1038/s41579-022-00846-2

DeCuir, J., Surie, D., Zhu, Y., Gaglani, M., Ginde, A. A., Douin, D. J., Talbot, K., Casey, J. D., Mohr, N. M., McNeal, T., Ghamande, S., Gibbs, K. W., Hager, D. N., Phan, M., Prekker, M. E., Gong, M. N., Mohamed, A., Johnson, N. J., Wilson, J. G., & Self, W. H. (2023). Effectiveness of Monovalent mRNA COVID-19 Vaccination in Preventing COVID-19–Associated Invasive Mechanical Ventilation and Death Among Immunocompetent Adults During the Omicron Variant Period — IVY Network, 19 U.S. States, February 1, 2022–January 31, 20. *MMWR Morb Mortal Wkly Rep*, *72*(17), 463–468.

Demko, Z. O., Yu, T., Mullapudi, S. K., Varela Heslin, M. G., Dorsey, C. A., Payton, C. B., Tornheim, J. A., Blair, P. W., Mehta, S. H., Thomas, D. L., Manabe, Y. C., & Antar, A. A. R. (2022). Post-acute sequelae of SARS-CoV-2 (PASC) impact quality of life at 6, 12 and 18 months post-infection. *MedRxiv*. https://doi.org/10.1101/2022.08.08.22278543

Deng, S., Liu, Y., Tam, R. C.-Y., Chen, P., Zhang, A. J., Mok, B. W.-Y., Long, T., Kukic, A., Zhou, R., Xu, H., Song, W., Chan, J. F.-W., To, K. K.-W., Chen, Z., Yuen, K.-Y., Wang, P., & Chen, H. (2023). An intranasal influenza virus-vectored vaccine prevents SARS-CoV-2 replication in respiratory tissues of mice and hamsters. *Nature Communications*, *14*(1), 2081. https://doi.org/10.1038/s41467-023-37697-1

Dhochak, N., Agrawal, T., Shaman, H., Khan, N. A., Kumar, P., Kabra, S. K., Medigeshi, G. R., & Lodha, R. (2022). Humoral cross-reactivity towards SARS-CoV-2 in young children with acute respiratory infection with low-pathogenicity coronaviruses. *Journal of Clinical Virology Plus*, *2*(1), 100061. https://doi.org/https://doi.org/10.1016/j.jcvp.2022.100061

Dobaño, C., Alonso, S., Fernández de Sevilla, M., Vidal, M., Jiménez, A., Pons Tomas, G., Jairoce, C., Melé Casas, M., Rubio, R., Hernández García, M., Ruiz-Olalla, G., Girona-Alarcón, M., Barrios, D., Santano, R., Mitchell, R. A., Puyol, L., Mayer, L., Chi, J., Rodrigo Melero, N., … Jordan, I. (2021). Antibody conversion rates to SARS-CoV-2 in saliva from children attending summer schools in Barcelona, Spain. *BMC Medicine*, *19*(1), 309. https://doi.org/10.1186/s12916-021-02184-1

Donnelly, C. A., Ghani, A. C., Leung, G. M., Hedley, A. J., Fraser, C., Riley, S., Abu-Raddad, L. J., Ho, L.-M., Thach, T.-Q., Chau, P., Chan, K.-P., Lam, T.-H., Tse, L.-Y., Tsang, T., Liu, S.-H., Kong, J. H. B., Lau, E. M. C., Ferguson, N. M., & Anderson, R. M. (2003). Epidemiological determinants of spread of causal agent of severe acute respiratory syndrome in Hong Kong. *The Lancet*, *361*(9371), 1761–1766. https://doi.org/https://doi.org/10.1016/S0140-6736(03)13410-1

Doria-Rose, N., Suthar, M. S., Makowski, M., O'Connell, S., McDermott, A. B., Flach, B., Ledgerwood, J. E., Mascola, J. R., Graham, B. S., Lin, B. C., O'Dell, S., Schmidt, S. D., Widge, A. T., Edara, V.-V., Anderson, E. J., Lai, L., Floyd, K., Rouphael, N. G., Zarnitsyna, V., … Kunwar, P. (2021). Antibody Persistence through 6 Months after the Second Dose of mRNA-1273 Vaccine for Covid-19. *New England Journal of Medicine*, *384*(23), 2259–2261. https://doi.org/10.1056/NEJMc2103916

Earnest, R., Uddin, R., Matluk, N., Renzette, N., Turbett, S. E., Siddle, K. J., Loreth, C., Adams, G., Tomkins-Tinch, C. H., Petrone, M. E., Rothman, J. E., Breban, M. I., Koch, R. T., Billig, K., Fauver, J. R., Vogels, C. B. F., Bilguvar, K., De Kumar, B., Landry, M. L., … Grubaugh, N. D. (2022). Comparative transmissibility of SARS-CoV-2 variants Delta and Alpha in New England, USA. *Cell Reports Medicine*, *3*(4), 100583. https://doi.org/https://doi.org/10.1016/j.xcrm.2022.100583

EMA. (2023). *COVID-19 vaccines*. https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines

European Comission. (2023). *COVID-19 In Vitro Diagnostic Medical Devices*. https://covid-19-diagnostics.jrc.ec.europa.eu/devices

Evans, S. J. W., & Jewell, N. P. (2021). Vaccine Effectiveness Studies in the Field. *New England Journal of Medicine*, *385*(7), 650–651. https://doi.org/10.1056/NEJMe2110605

Faria, N. R., Mellan, T. A., Whittaker, C., Claro, I. M., Candido, D. da S., Mishra, S., Crispim, M. A. E., Sales, F. C. S., Hawryluk, I., McCrone, J. T., Hulswit, R. J. G., Franco, L. A. M., Ramundo, M. S., de Jesus, J. G., Andrade, P. S., Coletti, T. M., Ferreira, G. M., Silva, C. A. M., Manuli, E. R., … Sabino, E. C. (2021). Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science (New York, N.Y.)*, *372*(6544), 815–821. https://doi.org/10.1126/science.abh2644

FDA. (2022). *In Vitro Diagnostics Emergency Use Authorizations (EUAs) - Serology and Other Adaptive Immune Response Tests for SARS-CoV-2*. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas-serology-and-other-adaptive-immune-response-tests-sars-cov-2

FDA. (2023a). *COVID-19 Vaccines*. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines#authorized-vaccines

FDA. (2023b). *Vaccines Licensed for Use in the United States | FDA*. U.S. Food and Drug Administration. https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states

Feikin, D. R., Higdon, M. M., Abu-Raddad, L. J., Andrews, N., Araos, R., Goldberg, Y., Groome, M. J., Huppert, A., O'Brien, K. L., Smith, P. G., Wilder-Smith, A., Zeger, S., Deloria Knoll, M., & Patel, M. K. (2022). Duration of effectiveness of vaccines against SARS-CoV-2 infection and COVID-19 disease: results of a systematic review and meta-regression. *Lancet*, *399*(10328), 924–944. https://doi.org/10.1016/S0140-6736(22)00152-0

Ferdinands, J. M., Gaglani, M., Martin, E. T., Middleton, D., Monto, A. S., Murthy, K., Silveira, F. P., Talbot, H. K., Zimmerman, R., Alyanak, E., Strickland, C., Spencer, S., & Fry, A. M. (2019). Prevention of Influenza Hospitalization Among Adults in the United States, 2015–2016: Results From the US Hospitalized Adult Influenza Vaccine Effectiveness Network (HAIVEN). *The Journal of Infectious Diseases*, *220*(8), 1265–1275. https://doi.org/10.1093/infdis/jiy723

Finnish Institute for Health and Welfare. (2021). *Coronavirus variants*. https://doi.org/https://thl.fi/en/web/infectious-diseases-and-vaccinations/what-s-new/coronavirus-covid-19-latest-updates/transmission-and-protection-coronavirus/coronavirus-variants

Finnish Institute for Health and Welfare. (2022). *THL recommends coronavirus vaccine booster for older people and risk groups this autumn - Press release*. https://thl.fi/en/web/thlfi-en/-/thl-recommends-coronavirus-vaccine-booster-for-older-people-and-risk-groups-this-autumn

Finnish Institute for Health and Welfare. (2023a). *Arranging COVID-19 vaccinations in Finland*. https://thl.fi/fi/web/infektiotaudit-ja-rokotukset/ajankohtaista/ajankohtaista-koronaviruksesta-covid-19/rokotteet-ja-koronavirus/koronarokotusten-jarjestaminen-suomessa

Finnish Institute for Health and Welfare. (2023b). *Study_ Coronavirus vaccines prevented around 7,300 deaths due to COVID-19 in Finland by the end of March 2022 - Press release*. https://thl.fi/en/web/thlfi-en/-/study-coronavirus-vaccines-prevented-around-7-300-deaths-due-to-covid-19-in-finland-by-the-end-of-march-2022

Finnish Institute for Health and Welfare. (2023c). *The progress of COVID-19 vaccination in Finland*. https://www.thl.fi/episeuranta/rokotukset/koronarokotusten_edistyminen.html

Gaebler, C., Wang, Z., Lorenzi, J. C. C., Muecksch, F., Finkin, S., Tokuyama, M., Cho, A., Jankovic, M., Schaefer-Babajew, D., Oliveira, T. Y., Cipolla, M., Viant, C., Barnes, C. O., Bram, Y., Breton, G., Hägglöf, T., Mendoza, P., Hurley, A., Turroja, M., … Nussenzweig, M. C. (2021). Evolution of antibody immunity to SARS-CoV-2. *Nature*, *591*(7851), 639–644. https://doi.org/10.1038/s41586-021-03207-w

Galani, I.-E., Rovina, N., Lampropoulou, V., Triantafyllia, V., Manioudaki, M., Pavlos, E., Koukaki, E., Fragkou, P. C., Panou, V., Rapti, V., Koltsida, O., Mentis, A., Koulouris, N., Tsiodras, S., Koutsoukou, A., & Andreakos, E. (2021). Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. *Nature Immunology*, *22*(1), 32–40. https://doi.org/10.1038/s41590-020-00840-x

Galipeau, Y., Siragam, V., Laroche, G., Marion, E., Greig, M., McGuinty, M., Booth, R. A., Durocher, Y., Cuperlovic-Culf, M., Bennett, S. A. L., Crawley, A. M., Giguère, P. M., Cooper, C., & Langlois, M.-A. (2021). Relative Ratios of Human Seasonal Coronavirus Antibodies Predict the Efficiency of Cross-Neutralization of SARS-CoV-2 Spike Binding to ACE2. *EBioMedicine*, *74*, 103700. https://doi.org/https://doi.org/10.1016/j.ebiom.2021.103700

GeurtsvanKessel, C. H., Geers, D., Schmitz, K. S., Mykytyn, A. Z., Lamers, M. M., Bogers, S., Scherbeijn, S., Gommers, L., Sablerolles, R. S. G., Nieuwkoop, N. N., Rijsbergen, L. C., van Dijk, L. L. A., de Wilde, J., Alblas, K., Breugem, T. I., Rijnders, B. J. A., de Jager, H., Weiskopf, D., van der Kuy, P. H. M., … de Vries, R. D. (2022). Divergent SARS-CoV-2 Omicron-reactive T and B cell responses in COVID-19 vaccine recipients. *Science Immunology*, *7*(69), eabo2202. https://doi.org/10.1126/sciimmunol.abo2202

GeurtsvanKessel, C. H., Okba, N. M. A., Igloi, Z., Bogers, S., Embregts, C. W. E., Laksono, B. M., Leijten, L., Rokx, C., Rijnders, B., Rahamat-Langendoen, J., van den Akker, J. P. C., van Kampen, J. J. A., van der Eijk, A. A., van Binnendijk, R. S., Haagmans, B., & Koopmans, M. (2020). An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. *Nature Communications*, *11*(1), 3436. https://doi.org/10.1038/s41467-020-17317-y

Goel, R. R., Apostolidis, S. A., Painter, M. M., Mathew, D., Pattekar, A., Kuthuru, O., Gouma, S., Hicks, P., Meng, W., Rosenfeld, A. M., Dysinger, S., Lundgreen, K. A., Kuri-Cervantes, L., Adamski, S., Hicks, A., Korte, S., Oldridge, D. A., Baxter, A. E., Giles, J. R., … Wherry, E. J. (2021). Distinct antibody and memory B cell responses in SARS-CoV-2 naive and recovered individuals following mRNA vaccination. *Science Immunology*, *6*(58). https://doi.org/10.1126/sciimmunol.abi6950

Goel, R. R., Painter, M. M., Apostolidis, S. A., Mathew, D., Meng, W., Rosenfeld, A. M., Lundgreen, K. A., Reynaldi, A., Khoury, D. S., Pattekar, A., Gouma, S., Kuri-Cervantes, L., Hicks, P., Dysinger, S., Hicks, A., Sharma, H., Herring, S., Korte, S., Baxter, A. E., … Wherry, E. J. (2023).

mRNA vaccines induce durable immune memory to SARS-CoV-2 and variants of concern. *Science*, *374*(6572), abm0829. https://doi.org/10.1126/science.abm0829

Goronzy, J. J., & Weyand, C. M. (2013). Understanding immunosenescence to improve responses to vaccines. *Nature Immunology*, *14*(5), 428–436. https://doi.org/10.1038/ni.2588

Graham, C., Seow, J., Huettner, I., Khan, H., Kouphou, N., Acors, S., Winstone, H., Pickering, S., Galao, R. P., Dupont, L., Lista, M. J., Jimenez-Guardeño, J. M., Laing, A. G., Wu, Y., Joseph, M., Muir, L., van Gils, M. J., Ng, W. M., Duyvesteyn, H. M. E., … Doores, K. J. (2021). Neutralization potency of monoclonal antibodies recognizing dominant and subdominant epitopes on SARS-CoV-2 Spike is impacted by the B.1.1.7 variant. *Immunity*, *54*(6), 1276-1289.e6. https://doi.org/10.1016/j.immuni.2021.03.023

Greaney, A. J., Starr, T. N., Gilchuk, P., Zost, S. J., Binshtein, E., Loes, A. N., Hilton, S. K., Huddleston, J., Eguia, R., Crawford, K. H. D., Dingens, A. S., Nargi, R. S., Sutton, R. E., Suryadevara, N., Rothlauf, P. W., Liu, Z., Whelan, S. P. J., Carnahan, R. H., Crowe, J. E., & Bloom, J. D. (2021). Complete Mapping of Mutations to the SARS-CoV-2 Spike Receptor-Binding Domain that Escape Antibody Recognition. *Cell Host & Microbe*, *29*(1), 44-57.e9. https://doi.org/https://doi.org/10.1016/j.chom.2020.11.007

Greinacher, A., Thiele, T., Warkentin, T. E., Weisser, K., Kyrle, P. A., & Eichinger, S. (2021). Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *New England Journal of Medicine*, *384*(22), 2092–2101. https://doi.org/10.1056/NEJMoa2104840

Grifoni, A., Weiskopf, D., Ramirez, S. I., Mateus, J., Dan, J. M., Moderbacher, C. R., Rawlings, S. A., Sutherland, A., Premkumar, L., Jadi, R. S., Marrama, D., de Silva, A. M., Frazier, A., Carlin, A. F., Greenbaum, J. A., Peters, B., Krammer, F., Smith, D. M., Crotty, S., & Sette, A. (2020). Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell*, *181*(7), 1489-1501.e15. https://doi.org/10.1016/j.cell.2020.05.015

Guan, Y., Zheng, B. J., He, Y. Q., Liu, X. L., Zhuang, Z. X., Cheung, C. L., Luo, S. W., Li, P. H., Zhang, L. J., Guan, Y. J., Butt, K. M., Wong, K. L., Chan, K. W., Lim, W., Shortridge, K. F., Yuen, K. Y., Peiris, J. S. M., & Poon, L. L. M. (2003). Isolation and Characterization of Viruses Related to the SARS Coronavirus from Animals in Southern China. *Science*, *302*(5643), 276–278. https://doi.org/10.1126/science.1087139

Guerrera, G., Picozza, M., D'Orso, S., Placido, R., Pirronello, M., Verdiani, A., Termine, A., Fabrizio, C., Giannessi, F., Sambucci, M., Balice, M. P., Caltagirone, C., Salvia, A., Rossini, A., Battistini, L., & Borsellino, G. (2023). BNT162b2 vaccination induces durable SARS-CoV-2–specific T cells with a stem cell memory phenotype. *Science Immunology*, *6*(66), eabl5344. https://doi.org/10.1126/sciimmunol.abl5344

Hachim, A., Kavian, N., Cohen, C. A., Chin, A. W. H., Chu, D. K. W., Mok, C. K. P., Tsang, O. T. Y., Yeung, Y. C., Perera, R. A. P. M., Poon, L. L. M., Peiris, J. S. M., & Valkenburg, S. A. (2020). ORF8 and ORF3b antibodies are accurate serological markers of early and late SARS-CoV-2 infection. *Nature Immunology*, *21*(10), 1293–1301. https://doi.org/10.1038/s41590-020-0773-7

Hamre, D., & Procknow, J. J. (1966). A New Virus Isolated from the Human Respiratory Tract. *Proceedings of the Society for Experimental Biology and Medicine*, *121*(1), 190–193. https://doi.org/10.3181/00379727-121-30734

Harari, S., Tahor, M., Rutsinsky, N., Meijer, S., Miller, D., Henig, O., Halutz, O., Levytskyi, K., Ben-Ami, R., Adler, A., Paran, Y., & Stern, A. (2022). Drivers of adaptive evolution during chronic SARS-CoV-2 infections. *Nature Medicine*, *28*(7), 1501–1508. https://doi.org/10.1038/s41591-022-01882-4

Hassan, A. O., Feldmann, F., Zhao, H., Curiel, D. T., Okumura, A., Tang-Huau, T.-L., Case, J. B., Meade-White, K., Callison, J., Chen, R. E., Lovaglio, J., Hanley, P. W., Scott, D. P., Fremont, D. H., Feldmann, H., & Diamond, M. S. (2021). A single intranasal dose of chimpanzee adenovirus-vectored vaccine protects against SARS-CoV-2 infection in rhesus macaques. *Cell Reports Medicine*, *2*(4), 100230. https://doi.org/https://doi.org/10.1016/j.xcrm.2021.100230

Hassanin, A., Rambaud, O., & Klein, D. (2022). Genomic Bootstrap Barcodes and Their Application to Study the Evolution of Sarbecoviruses. *Viruses*, *14*(2). https://doi.org/10.3390/v14020440

Haveri, A., Ekström, N., Solastie, A., Virta, C., Österlund, P., Isosaari, E., Nohynek, H., Palmu, A. A., & Melin, M. (2021). Persistence of neutralizing antibodies a year after SARS-CoV-2 infection in humans. *European Journal of Immunology*, *51*, 3202–3213. https://doi.org/https://doi.org/10.1002/eji.202149535

Havers, F. P., Patel, K., Whitaker, M., Milucky, J., Reingold, A., Armistead, I., Meek, J., Anderson, E. J., Weigel, A., Reeg, L., Seys, S., Ropp, S. L., Spina, N., Felsen, C. B., Moran, N. E., Sutton, M., Talbot, K., George, A., & Taylor, C. A. (2022). Laboratory-Confirmed COVID-19 – Associated Hospitalizations Among Adults During SARS-CoV-2 Omicron BA . 2 Variant Predominance — COVID-19 – Associated Hospitalization Surveillance Network , 14 States, June 20, 2021–May 31, 2022. *MMWR Morb Mortal Wkly Rep*, *71*(34), 1085–1091. https://doi.org/http://dx.doi.org/10.15585/mmwr.mm7134a3

He, J.-F., Peng, G.-W., Min, J., Y, D.-W., Liang, W.-J., Zhang, S.-Y., Xu, R.-H., Zheng, H.-Y., Wu, X.-W., Xu, J., Wang, Z.-H., Fang, L., Zhang, X., Li, H., Yan, X.-G., Lu, J.-H., Hu, Z.-H., Huang, J.-C., Wan, Z.-Y., … Lo, Y. M. D. (2004). Molecular Evolution of the SARS Coronavirus During the Course of the SARS Epidemic in China. *Science*, *303*(5664), 1666–1669. https://doi.org/10.1126/science.1092002

He, J., Guo, Y., Mao, R., & Zhang, J. (2021). Proportion of asymptomatic coronavirus disease 2019: A systematic review and meta-analysis. *Journal of Medical Virology*, *93*(2), 820–830. https://doi.org/https://doi.org/10.1002/jmv.26326

Henao-Restrepo, A. M., Camacho, A., Longini, I. M., Watson, C. H., Edmunds, W. J., Egger, M., Carroll, M. W., Dean, N. E., Diatta, I., Doumbia, M., Draguez, B., Duraffour, S., Enwere, G., Grais, R., Gunther, S., Gsell, P.-S., Hossmann, S., Watle, S. V., Kondé, M. K., … Kieny, M.-P. (2017). Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). *Lancet (London, England)*, *389*(10068), 505–518. https://doi.org/10.1016/S0140-6736(16)32621-6

Henderson, D. K., Weber, D. J., Babcock, H., Hayden, M. K., Malani, A., Wright, S. B., Murthy, A. R., Guzman-Cottrill, J., Haessler, S., Rock, C., Van Schooneveld, T., Logan, L., & Forde, C. (2021). The perplexing problem of persistently PCR-positive personnel. *Infection Control & Hospital Epidemiology*, *42*(2), 203–204. https://doi.org/DOI: 10.1017/ice.2020.343

Hicks, J., Klumpp-Thomas, C., Kalish, H., Shunmugavel, A., Mehalko, J., Denson, J.-P., Snead, K. R., Drew, M., Corbett, K. S., Graham, B. S., Hall, M. D., Memoli, M. J., Esposito, D., & Sadtler, K. (2021). Serologic Cross-Reactivity of SARS-CoV-2 with Endemic and Seasonal Betacoronaviruses. *Journal of Clinical Immunology*, *41*(5), 906–913. https://doi.org/10.1007/s10875-021-00997-6

Huang, Y., Yang, C., Xu, X., Xu, W., & Liu, S. (2020). Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacologica Sinica*, *41*(9), 1141–1149. https://doi.org/10.1038/s41401-020-0485-4

Imai, M., Iwatsuki-Horimoto, K., Hatta, M., Loeber, S., Halfmann, P. J., Nakajima, N., Watanabe, T., Ujie, M., Takahashi, K., Ito, M., Yamada, S., Fan, S., Chiba, S., Kuroda, M., Guan, L., Takada, K., Armbrust, T., Balogh, A., Furusawa, Y., … Kawaoka, Y. (2020). Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *PNAS*, *117*(28), 16587–16595. https://doi.org/10.1073/pnas.2009799117

Isho, B., Abe, K. T., Zuo, M., Jamal, A. J., Rathod, B., Wang, J. H., Li, Z., Chao, G., Rojas, O. L., Bang, Y. M., Pu, A., Christie-Holmes, N., Gervais, C., Ceccarelli, D., Samavarchi-Tehrani, P., Guvenc, F., Budylowski, P., Li, A., Paterson, A., … Gingras, A.-C. (2020). Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Science Immunology*, *5*(52), eabe5511. https://doi.org/10.1126/sciimmunol.abe5511

Iyer, A. S., Jones, F. K., Nodoushani, A., Kelly, M., Becker, M., Slater, D., Mills, R., Teng, E., Kamruzzaman, M., Garcia-Beltran, W. F., Astudillo, M., Yang, D., Miller, T. E., Oliver, E., Fischinger, S., Atyeo, C., Iafrate, A. J., Calderwood, S. B., Lauer, S. A., … Charles, R. C. (2020). Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Science Immunology*, *5*(52), 1–13. doi:10.1126/sciimmunol.abe0367

Jääskeläinen, A. J., Kuivanen, S., Kekäläinen, E., Ahava, M. J., Loginov, R., Kallio-Kokko, H., Vapalahti, O., Jarva, H., Kurkela, S., & Lappalainen, M. (2020). Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. *Journal of Clinical Virology*, *129*, 104512. https://doi.org/https://doi.org/10.1016/j.jcv.2020.104512

Jackson, N. A. C., Kester, K. E., Casimiro, D., Gurunathan, S., & DeRosa, F. (2020). The promise of mRNA vaccines: a biotech and industrial perspective. *Npj Vaccines*, *5*(1), 11. https://doi.org/10.1038/s41541-020-0159-8

Janeway CA Jr, Travers P, Walport M. (2001). The distribution and functions of immunoglobulin isotypes. In *Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science.*

Jernej, P., Julia, K., Karola, M., Enrico, R., Jasmin, Z., Hannah, P., Bianca, S., Galit, A., & Hendrik, S. (2022). Persistent Maintenance of Intermediate Memory B Cells Following SARS-CoV-2 Infection and Vaccination Recall Response. *Journal of Virology*, *96*(15), e00760-22. https://doi.org/10.1128/jvi.00760-22

Jose, L. M., Helena, W., D., W. H., Adam, D., Suzanne, P., Pedro, G. R., Giuditta, D. L., M., C. V., Wilhelm, F., Nicolas, S., Richard, O., Massimo, P., H., P. A., Luke, S., Gaia, N., Chad, S., & D., N. S. J. (2022). The P681H Mutation in the Spike Glycoprotein of the Alpha Variant of SARS-CoV-2 Escapes IFITM Restriction and Is Necessary for Type I Interferon Resistance. *Journal of Virology*, *96*(23), e01250-22. https://doi.org/10.1128/jvi.01250-22

Kaech, S. M., Wherry, E. J., & Ahmed, R. (2002). Effector and memory T-cell differentiation: implications for vaccine development. *Nature Reviews Immunology*, *2*(4), 251–262. https://doi.org/10.1038/nri778

Kalimuddin, S., Tham, C. Y. L., Qui, M., de Alwis, R., Sim, J. X. Y., Lim, J. M. E., Tan, H.-C., Syenina, A., Zhang, S. L., Le Bert, N., Tan, A. T., Leong, Y. S., Yee, J. X., Ong, E. Z., Ooi, E. E., Bertoletti, A., & Low, J. G. (2021). Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. *Med*, *2*(6), 682-688.e4. https://doi.org/https://doi.org/10.1016/j.medj.2021.04.003

Kan, B., Wang, M., Jing, H., Xu, H., Jiang, X., Yan, M., Liang, W., Zheng, H., Wan, K., Liu, Q., Cui, B., Xu, Y., Zhang, E., Wang, H., Ye, J., Li, G., Li, M., Cui, Z., Qi, X., … Xu, J. (2005). Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms. *Journal of Virology*, *79*(18), 11892–11900. https://doi.org/10.1128/JVI.79.18.11892-11900.2005

Kaplonek, P., Wang, C., Bartsch, Y., Fischinger, S., Gorman, M. J., Bowman, K., Kang, J., Dayal, D., Martin, P., Nowak, R. P., Villani, A.-C., Hsieh, C.-L., Charland, N. C., Gonye, A. L. K., Gushterova, I., Khanna, H. K., LaSalle, T. J., Lavin-Parsons, K. M., Lilley, B. M., … Alter, G. (2023). Early cross-coronavirus reactive signatures of humoral immunity against COVID-19. *Science Immunology*, *6*(64), eabj2901. https://doi.org/10.1126/sciimmunol.abj2901

Karlstad, Ø., Hovi, P., Husby, A., Härkänen, T., Selmer, R. M., Pihlström, N., Hansen, J. V., Nohynek, H., Gunnes, N., Sundström, A., Wohlfahrt, J., Nieminen, T. A., Grünewald, M., Gulseth, H. L., Hviid, A., & Ljung, R. (2022). SARS-CoV-2 Vaccination and Myocarditis in a Nordic Cohort Study of 23 Million Residents. *JAMA Cardiology*, *7*(6), 600–612. https://doi.org/10.1001/jamacardio.2022.0583

Kazakova, A., Kakkola, L., Päkkilä, H., Teros-Jaakkola, T., Soukka, T., Peltola, V., Waris, M., & Julkunen, I. (2019). Serological Array-in-Well Multiplex Assay Reveals a High Rate of

Respiratory Virus Infections and Reinfections in Young Children. *MSphere*, *4*(5), 1–14. https://doi.org/10.1128/msphere.00447-19

Kellam, P., & Barclay, W. (2020). The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. *Journal of General Virology*, *101*(8), 791–797. https://doi.org/https://doi.org/10.1099/jgv.0.001439

Khoury, D. S., Cromer, D., Reynaldi, A., Schlub, T. E., Wheatley, A. K., Juno, J. A., Subbarao, K., Kent, S. J., Triccas, J. A., & Davenport, M. P. (2021). Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Medicine*, *27*(7), 1205–1211. https://doi.org/10.1038/s41591-021-01377-8

Kim, M.-C., Cui, C., Shin, K.-R., Bae, J.-Y., Kweon, O.-J., Lee, M.-K., Choi, S.-H., Jung, S.-Y., Park, M.-S., & Chung, J.-W. (2021). Duration of Culturable SARS-CoV-2 in Hospitalized Patients with Covid-19. *New England Journal of Medicine*, *384*(7), 671–673. https://doi.org/10.1056/NEJMc2027040

Kolehmainen, P., Huttunen, M., Iakubovskaia, A., Maljanen, S., Tauriainen, S., Yatkin, E., Pasternack, A., Naves, R., Toivonen, L., Tähtinen, P. A., Ivaska, L., Lempainen, J., Peltola, V., Waris, M., Kakkola, L., Ritvos, O., & Julkunen, I. (2023). Coronavirus spike protein - specific antibodies indicate frequent infections and reinfections in infancy and among BNT162b2 - vaccinated healthcare workers. *Scientific Reports*, 1–14. https://doi.org/10.1038/s41598-023-35471-3

Korosec, C. S., Farhang-Sardroodi, S., Dick, D. W., Gholami, S., Ghaemi, M. S., Moyles, I. R., Craig, M., Ooi, H. K., & Heffernan, J. M. (2022). Long-term durability of immune responses to the BNT162b2 and mRNA-1273 vaccines based on dosage, age and sex. *Scientific Reports*, *12*(1), 21232. https://doi.org/10.1038/s41598-022-25134-0

Koutsakos, M., Reynaldi, A., Lee, W. S., Nguyen, J., Amarasena, T., Taiaroa, G., Kinsella, P., Liew, K. C., Tran, T., Kent, H. E., Tan, H.-X., Rowntree, L. C., Nguyen, T. H. O., Thomas, P. G., Kedzierska, K., Petersen, J., Rossjohn, J., Williamson, D. A., Khoury, D., … Juno, J. A. (2023). SARS-CoV-2 breakthrough infection induces rapid memory and de novo T cell responses. *Immunity*, *56*(4), 879-892.e4. https://doi.org/https://doi.org/10.1016/j.immuni.2023.02.017

Krammer, F. (2019). The human antibody response to influenza A virus infection and vaccination. *Nature Reviews Immunology*, *19*(6), 383–397. https://doi.org/10.1038/s41577-019-0143-6

Kuchipudi, S. V, Surendran-Nair, M., Ruden, R. M., Yon, M., Nissly, R. H., Vandegrift, K. J., Nelli, R. K., Li, L., Jayarao, B. M., Maranas, C. D., Levine, N., Willgert, K., Conlan, A. J. K., Olsen, R. J., Davis, J. J., Musser, J. M., Hudson, P. J., & Kapur, V. (2022). Multiple spillovers from humans and onward transmission of SARS-CoV-2 in white-tailed deer. *Proceedings of the National Academy of Sciences*, *119*(6), e2121644119. https://doi.org/10.1073/pnas.2121644119

Kundu, R., Narean, J. S., Wang, L., Fenn, J., Pillay, T., Fernandez, N. D., Conibear, E., Koycheva, A., Davies, M., Tolosa-Wright, M., Hakki, S., Varro, R., McDermott, E., Hammett, S., Cutajar, J., Thwaites, R. S., Parker, E., Rosadas, C., McClure, M., … Lalvani, A. (2022). Cross-reactive memory T cells associate with protection against SARS-CoV-2 infection in COVID-19 contacts. *Nature Communications*, *13*(1), 80. https://doi.org/10.1038/s41467-021-27674-x

Lagerqvist, N., Maleki, K. T., Verner-Carlsson, J., Olausson, M., Dillner, J., Wigren Byström, J., Monsen, T., Forsell, M., Eriksson, J., Bogdanovic, G., Muschiol, S., Ljunggren, J., Repo, J., Kjerstadius, T., Muradrasoli, S., Brytting, M., Szekely Björndal, Å., Åkerlund, T., Nilsson, C., & Klingström, J. (2021). Evaluation of 11 SARS-CoV-2 antibody tests by using samples from patients with defined IgG antibody titers. *Scientific Reports*, *11*(1), 7614. https://doi.org/10.1038/s41598-021-87289-6

Laidlaw, B. J., Craft, J. E., & Kaech, S. M. (2016). The multifaceted role of CD4+ T cells in CD8+ T cell memory. *Nature Reviews Immunology*, *16*(2), 102–111. https://doi.org/10.1038/nri.2015.10

Laidlaw, B. J., Zhang, N., Marshall, H. D., Staron, M. M., Guan, T., Hu, Y., Cauley, L. S., Craft, J., & Kaech, S. M. (2014). CD4+ T Cell Help Guides Formation of CD103+ Lung-Resident Memory CD8+ T Cells during Influenza Viral Infection. *Immunity*, *41*(4), 633–645. https://doi.org/https://doi.org/10.1016/j.immuni.2014.09.007

Le Bert, N., Tan, A. T., Kunasegaran, K., Tham, C. Y. L., Hafezi, M., Chia, A., Chng, M. H. Y., Lin, M., Tan, N., Linster, M., Chia, W. N., Chen, M. I.-C., Wang, L.-F., Ooi, E. E., Kalimuddin, S., Tambyah, P. A., Low, J. G.-H., Tan, Y.-J., & Bertoletti, A. (2020). SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature*, *584*(7821), 457–462. https://doi.org/10.1038/s41586-020-2550-z

Lechien, J. R., Chiesa-Estomba, C. M., Place, S., Van Laethem, Y., Cabaraux, P., Mat, Q., Huet, K., Plzak, J., Horoi, M., Hans, S., Rosaria Barillari, M., Cammaroto, G., Fakhry, N., Martiny, D., Ayad, T., Jouffe, L., Hopkins, C., Saussez, S., & YO-IFOS, C.-19 T. F. of. (2020). Clinical and epidemiological characteristics of 1420 European patients with mild-to-moderate coronavirus disease 2019. *Journal of Internal Medicine*, *288*(3), 335–344. https://doi.org/https://doi.org/10.1111/joim.13089

Leiner, J., Pellissier, V., Hohenstein, S., König, S., Schuler, E., Möller, R., Nachtigall, I., Bonsignore, M., Hindricks, G., Kuhlen, R., & Bollmann, A. (2022). Characteristics and outcomes of COVID-19 patients during B.1.1.529 (Omicron) dominance compared to B.1.617.2 (Delta) in 89 German hospitals. *BMC Infectious Diseases*, *22*(1), 802. https://doi.org/10.1186/s12879-022-07781-w

Lim, J. M. E., Tan, A. T., Le Bert, N., Hang, S. K., Low, J. G. H., & Bertoletti, A. (2022). SARS-CoV-2 breakthrough infection in vaccinees induces virus-specific nasal-resident CD8+ and CD4+ T cells of broad specificity. *The Journal of Experimental Medicine*, *219*(10). https://doi.org/10.1084/jem.20220780

Lin, L., Liu, Y., Tang, X., & He, D. (2021). The Disease Severity and Clinical Outcomes of the SARS-CoV-2 Variants of Concern. *Frontiers in Public Health*, *9*. https://doi.org/10.3389/fpubh.2021.775224

Lisboa Bastos, M., Tavaziva, G., Abidi, S. K., Campbell, J. R., Haraoui, L.-P., Johnston, J. C., Lan, Z., Law, S., MacLean, E., Trajman, A., Menzies, D., Benedetti, A., & Ahmad Khan, F. (2020). Diagnostic accuracy of serological tests for covid-19: systematic review and meta-analysis. *BMJ (Clinical Research Ed.)*, *370*, m2516. https://doi.org/10.1136/bmj.m2516

Liu, C., Ginn, H. M., Dejnirattisai, W., Supasa, P., Wang, B., Tuekprakhon, A., Nutalai, R., Zhou, D., Mentzer, A. J., Zhao, Y., Duyvesteyn, H. M. E., López-Camacho, C., Slon-Campos, J., Walter, T. S., Skelly, D., Johnson, S. A., Ritter, T. G., Mason, C., Costa Clemens, S. A., … Screaton, G. R. (2021). Reduced neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell*, *184*(16), 4220-4236.e13. https://doi.org/https://doi.org/10.1016/j.cell.2021.06.020

Liu, H., Zhang, Q., Wei, P., Chen, Z., Aviszus, K., Yang, J., Downing, W., Jiang, C., Liang, B., Reynoso, L., Downey, G. P., Frankel, S. K., Kappler, J., Marrack, P., & Zhang, G. (2021). The basis of a more contagious 501Y.V1 variant of SARS-CoV-2. *Cell Research*, *31*(6), 720–722. https://doi.org/10.1038/s41422-021-00496-8

Liu, L., Wang, P., Nair, M. S., Yu, J., Rapp, M., Wang, Q., Luo, Y., Chan, J. F.-W., Sahi, V., Figueroa, A., Guo, X. V, Cerutti, G., Bimela, J., Gorman, J., Zhou, T., Chen, Z., Yuen, K.-Y., Kwong, P. D., Sodroski, J. G., … Ho, D. D. (2020). Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. *Nature*, *584*(7821), 450–456. https://doi.org/10.1038/s41586-020-2571-7

Liu, Y., Liu, J., Johnson, B. A., Xia, H., Ku, Z., Schindewolf, C., Widen, S. G., An, Z., Weaver, S. C., Menachery, V. D., Xie, X., & Shi, P.-Y. (2022). Delta spike P681R mutation enhances SARS-CoV-2 fitness over Alpha variant. *Cell Reports*, *39*(7). https://doi.org/10.1016/j.celrep.2022.110829

Long, Q.-X., Liu, B.-Z., Deng, H.-J., Wu, G.-C., Deng, K., Chen, Y.-K., Liao, P., Qiu, J.-F., Lin, Y., Cai, X.-F., Wang, D.-Q., Hu, Y., Ren, J.-H., Tang, N., Xu, Y.-Y., Yu, L.-H., Mo, Z., Gong, F., Zhang, X.-L., … Huang, A.-L. (2020). Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nature Medicine*, *26*(6), 845–848. https://doi.org/10.1038/s41591-020-0897-1

Lopez Bernal, J., Andrews, N., Gower, C., Gallagher, E., Simmons, R., Thelwall, S., Stowe, J., Tessier, E., Groves, N., Dabrera, G., Myers, R., Campbell, C. N. J., Amirthalingam, G., Edmunds, M., Zambon, M., Brown, K. E., Hopkins, S., Chand, M., & Ramsay, M. (2021). Effectiveness of

Covid-19 Vaccines against the B.1.617.2 (Delta) Variant. *New England Journal of Medicine*. https://doi.org/10.1056/NEJMoa2108891

Lou, B., Li, T.-D., Zheng, S.-F., Su, Y.-Y., Li, Z.-Y., Liu, W., Yu, F., Ge, S.-X., Zou, Q.-D., Yuan, Q., Lin, S., Hong, C.-M., Yao, X.-Y., Zhang, X.-J., Wu, D.-H., Zhou, G.-L., Hou, W.-H., Li, T.-T., Zhang, Y.-L., … Chen, Y. (2020). Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. *European Respiratory Journal*, *56*(2), 2000763. https://doi.org/10.1183/13993003.00763-2020

Lucas, C., Klein, J., Sundaram, M. E., Liu, F., Wong, P., Silva, J., Mao, T., Oh, J. E., Mohanty, S., Huang, J., Tokuyama, M., Lu, P., Venkataraman, A., Park, A., Israelow, B., Vogels, C. B. F., Muenker, M. C., Chang, C.-H., Casanovas-Massana, A., … Iwasaki, A. (2021). Delayed production of neutralizing antibodies correlates with fatal COVID-19. *Nature Medicine*, *27*(7), 1178–1186. https://doi.org/10.1038/s41591-021-01355-0

Lumley, S. F., Wei, J., Odonnell, D., Stoesser, N. E., Matthews, P. C., Howarth, A., Hatch, S. B., Marsden, B. D., Cox, S., James, T., Peck, L. J., Ritter, T. G., De Toledo, Z., Cornall, R. J., Jones, E. Y., Stuart, D. I., Screaton, G., Ebner, D., Hoosdally, S., … Eyre, D. W. (2021). The Duration, Dynamics, and Determinants of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibody Responses in Individual Healthcare Workers. *Clinical Infectious Diseases*, *73*(3), E699–E709. https://doi.org/10.1093/cid/ciab004

Lustig, Y., Nemet, I., Kliker, L., Zuckerman, N., Yishai, R., Alroy-Preis, S., Mendelson, E., & Mandelboim, M. (2021). Neutralizing Response against Variants after SARS-CoV-2 Infection and One Dose of BNT162b2. *New England Journal of Medicine*, *384*(25), 2453–2454. https://doi.org/10.1056/NEJMc2104036

Ma, H., Zeng, W., He, H., Zhao, D., Jiang, D., Zhou, P., Cheng, L., Li, Y., Ma, X., & Jin, T. (2020). Serum IgA, IgM, and IgG responses in COVID-19. *Cellular & Molecular Immunology*, *17*(7), 773–775. https://doi.org/10.1038/s41423-020-0474-z

Ma, Q., Liu, J., Liu, Q., Kang, L., Liu, R., Jing, W., Wu, Y., & Liu, M. (2021). Global Percentage of Asymptomatic SARS-CoV-2 Infections Among the Tested Population and Individuals With Confirmed COVID-19 Diagnosis: A Systematic Review and Meta-analysis. *JAMA Network Open*, *4*(12), e2137257–e2137257. https://doi.org/10.1001/jamanetworkopen.2021.37257

Macedo, A. C. L., Prestes, G. da S., Colonetti, T., Candido, A. C. R., Uggioni, M. L. R., Gomes, A. C., Silva, F. R., Ceretta, L. B., Grande, A. J., & da Rosa, M. I. (2022). A systematic review and meta-analysis of the accuracy of SARS-COV-2 IgM and IgG tests in individuals with COVID-19. *Journal of Clinical Virology*, *148*, 105121. https://doi.org/10.1016/j.jcv.2022.105121

Madhavan, M., Ritchie, A. J., Aboagye, J., Jenkin, D., Provstgaad-Morys, S., Tarbet, I., Woods, D., Davies, S., Baker, M., Platt, A., Flaxman, A., Smith, H., Belij-Rammerstorfer, S., Wilkins, D., Kelly, E. J., Villafana, T., Green, J. A., Poulton, I., Lambe, T., … Douglas, A. D. (2022). Tolerability and immunogenicity of an intranasally-administered adenovirus-vectored COVID-19 vaccine: An open-label partially-randomised ascending dose phase I trial. *EBioMedicine*, *85*. https://doi.org/10.1016/j.ebiom.2022.104298

Marcotte, H., Piralla, A., Zuo, F., Du, L., Cassaniti, I., Wan, H., Kumagai-Braesh, M., Andréll, J., Percivalle, E., Sammartino, J. C., Wang, Y., Vlachiotis, S., Attevall, J., Bergami, F., Ferrari, A., Colaneri, M., Vecchia, M., Sambo, M., Zuccaro, V., … Pan-Hammarström, Q. (2022). Immunity to SARS-CoV-2 up to 15 months after infection. *IScience*, *25*(2), 103743. https://doi.org/https://doi.org/10.1016/j.isci.2022.103743

Maringer, Y., Nelde, A., Schroeder, S. M., Schuhmacher, J., Hörber, S., Peter, A., Karbach, J., Jäger, E., & Walz, J. S. (2023). Durable spike-specific T cell responses after different COVID-19 vaccination regimens are not further enhanced by booster vaccination. *Science Immunology*, *7*(78), eadd3899. https://doi.org/10.1126/sciimmunol.add3899

Markov, P. V, Ghafari, M., Beer, M., Lythgoe, K., Simmonds, P., Stilianakis, N. I., & Katzourakis, A. (2023). The evolution of SARS-CoV-2. *Nature Reviews Microbiology*, *21*(6), 361–379. https://doi.org/10.1038/s41579-023-00878-2

Martin, D. P., Lytras, S., Lucaci, A. G., Maier, W., Grüning, B., Shank, S. D., Weaver, S., MacLean, O. A., Orton, R. J., Lemey, P., Boni, M. F., Tegally, H., Harkins, G. W., Scheepers, C., Bhiman, J. N., Everatt, J., Amoako, D. G., San, J. E., Giandhari, J., … Kosakovsky Pond, S. L. (2022). Selection Analysis Identifies Clusters of Unusual Mutational Changes in Omicron Lineage BA.1 That Likely Impact Spike Function. *Molecular Biology and Evolution*, *39*(4), msac061. https://doi.org/10.1093/molbev/msac061

Mathew, D., Giles, J. R., Baxter, A. E., Oldridge, D. A., Greenplate, A. R., Wu, J. E., Alanio, C., Kuri-Cervantes, L., Pampena, M. B., D'Andrea, K., Manne, S., Chen, Z., Huang, Y. J., Reilly, J. P., Weisman, A. R., Ittner, C. A. G., Kuthuru, O., Dougherty, J., Nzingha, K., … Wherry, E. J. (2020). Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science*, *369*(6508), eabc8511. https://doi.org/10.1126/science.abc8511

McEllistrem, M. C., Clancy, C. J., Buehrle, D. J., Singh, N., Lucas, A., Sirianni, V., & Decker, B. K. (2021). SARS-CoV-2 is associated with high viral loads in asymptomatic and recently symptomatic healthcare workers. *PloS One*, *16*(3), e0248347. https://doi.org/10.1371/journal.pone.0248347

McGill COVID19 Vaccine Tracker Team. (2022). *COVID-19 Vaccine Development and Approvals Tracker*. https://covid19.trackvaccines.org/site-credits/

McIntosh, K., Dees, J. H., Becker, W. B., Kapikian, A. Z., & Chanock, R. M. (1967). Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proceedings of the National Academy of Sciences*, *57*(4), 933–940. https://doi.org/10.1073/pnas.57.4.933

McKinstry, K. K., Strutt, T. M., Kuang, Y., Brown, D. M., Sell, S., Dutton, R. W., & Swain, S. L. (2012). Memory CD4+ T cells protect against influenza through multiple synergizing mechanisms. *The Journal of Clinical Investigation*, *122*(8), 2847–2856. https://doi.org/10.1172/JCI63689

McMahan, K., Yu, J., Mercado, N. B., Loos, C., Tostanoski, L. H., Chandrashekar, A., Liu, J., Peter, L., Atyeo, C., Zhu, A., Bondzie, E. A., Dagotto, G., Gebre, M. S., Jacob-Dolan, C., Li, Z., Nampanya, F., Patel, S., Pessaint, L., Van Ry, A., … Barouch, D. H. (2021). Correlates of protection against SARS-CoV-2 in rhesus macaques. *Nature*, *590*(7847), 630–634. https://doi.org/10.1038/s41586-020-03041-6

McMillen, T., Jani, K., Robilotti, E. V, Kamboj, M., & Babady, N. E. (2022). The spike gene target failure (SGTF) genomic signature is highly accurate for the identification of Alpha and Omicron SARS-CoV-2 variants. *Scientific Reports*, *12*(1), 18968. https://doi.org/10.1038/s41598-022-21564-y

Meng, B., Kemp, S. A., Papa, G., Datir, R., Ferreira, I. A. T. M., Marelli, S., Harvey, W. T., Lytras, S., Mohamed, A., Gallo, G., Thakur, N., Collier, D. A., Mlcochova, P., Robson, S. C., Loman, N. J., Connor, T. R., Golubchik, T., Martinez Nunez, R. T., Ludden, C., … Gupta, R. K. (2021). Recurrent emergence of SARS-CoV-2 spike deletion H69/V70 and its role in the Alpha variant B.1.1.7. *Cell Reports*, *35*(13). https://doi.org/10.1016/j.celrep.2021.109292

Min, Y.-Q., Huang, M., Sun, X., Deng, F., Wang, H., & Ning, Y.-J. (2021). Immune evasion of SARS-CoV-2 from interferon antiviral system. *Computational and Structural Biotechnology Journal*, *19*, 4217–4225. https://doi.org/https://doi.org/10.1016/j.csbj.2021.07.023

Minervina, A. A., Pogorelyy, M. V, Kirk, A. M., Crawford, J. C., Allen, E. K., Chou, C.-H., Mettelman, R. C., Allison, K. J., Lin, C.-Y., Brice, D. C., Zhu, X., Vegesana, K., Wu, G., Trivedi, S., Kottapalli, P., Darnell, D., McNeely, S., Olsen, S. R., Schultz-Cherry, S., … Thomas, P. G. (2022). SARS-CoV-2 antigen exposure history shapes phenotypes and specificity of memory CD8(+) T cells. *Nature Immunology*, *23*(5), 781–790. https://doi.org/10.1038/s41590-022-01184-4

Mitchell, S. L., Orris, S., Freeman, T., Freeman, M. C., Adam, M., Axe, M., Gribschaw, J., Suyama, J., Hoberman, A., & Wells, A. (2021). Performance of SARS-CoV-2 antigen testing in symptomatic and asymptomatic adults: a single-center evaluation. *BMC Infectious Diseases*, *21*(1), 1071. https://doi.org/10.1186/s12879-021-06716-1

Mlcochova, P., Kemp, S. A., Dhar, M. S., Papa, G., Meng, B., Ferreira, I. A. T. M., Datir, R., Collier, D. A., Albecka, A., Singh, S., Pandey, R., Brown, J., Zhou, J., Goonawardane, N., Mishra, S., Whittaker, C., Mellan, T., Marwal, R., Datta, M., … Gupta, R. K. (2021). SARS-CoV-2 B.1.617.2 Delta variant replication and immune evasion. *Nature*, *599*(7883), 114–119. https://doi.org/10.1038/s41586-021-03944-y

Mohd, H. A., Al-Tawfiq, J. A., & Memish, Z. A. (2016). Middle East Respiratory Syndrome Coronavirus (MERS-CoV) origin and animal reservoir. *Virology Journal*, *13*(1), 87. https://doi.org/10.1186/s12985-016-0544-0

Moran, T. M., Park, H., Fernandez-Sesma, A., & Schulman, J. L. (1999). Th2 Responses to Inactivated Influenza Virus Can Be Converted to Th1 Responses and Facilitate Recovery from Heterosubtypic Virus Infection. *The Journal of Infectious Diseases*, *180*(3), 579–585. https://doi.org/10.1086/314952

Moss, P. (2022). The T cell immune response against SARS-CoV-2. *Nature Immunology*, *23*(2), 186–193. https://doi.org/10.1038/s41590-021-01122-w

Muecksch, F., Weisblum, Y., Barnes, C. O., Schmidt, F., Schaefer-Babajew, D., Wang, Z., C. Lorenzi, J. C., Flyak, A. I., DeLaitsch, A. T., Huey-Tubman, K. E., Hou, S., Schiffer, C. A., Gaebler, C., Da Silva, J., Poston, D., Finkin, S., Cho, A., Cipolla, M., Oliveira, T. Y., … Bieniasz, P. D. (2021). Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. *Immunity*, *54*(8), 1853-1868.e7. https://doi.org/https://doi.org/10.1016/j.immuni.2021.07.008

Naaber, P., Tserel, L., Kangro, K., Sepp, E., Jürjenson, V., Adamson, A., Haljasmägi, L., Rumm, A. P., Maruste, R., Kärner, J., Gerhold, J. M., Planken, A., Ustav, M., Kisand, K., & Peterson, P. (2021). Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. *The Lancet Regional Health – Europe*, *10*. https://doi.org/10.1016/j.lanepe.2021.100208

Naranbhai, V., Nathan, A., Kaseke, C., Berrios, C., Khatri, A., Choi, S., Getz, M. A., Tano-Menka, R., Ofoman, O., Gayton, A., Senjobe, F., Zhao, Z., St Denis, K. J., Lam, E. C., Carrington, M., Garcia-Beltran, W. F., Balazs, A. B., Walker, B. D., Iafrate, A. J., & Gaiha, G. D. (2022). T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. *Cell*, *185*(6), 1041-1051.e6. https://doi.org/10.1016/j.cell.2022.01.029

Nasreen, S., Chung, H., He, S., Brown, K. A., Gubbay, J. B., Buchan, S. A., Fell, D. B., Austin, P. C., Schwartz, K. L., Sundaram, M. E., Calzavara, A., Chen, B., Tadrous, M., Wilson, K., Wilson, S. E., & Kwong, J. C. (2022). Effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection and severe outcomes with variants of concern in Ontario. *Nature Microbiology*, *7*(3), 379–385. https://doi.org/10.1038/s41564-021-01053-0

Nathalie, R., Soizic, D., Nadège, C., Matthieu, P., Frédérique, R., Sylvie, P., Julien, L., Carole, T., Catherine, P., Guillaume, G., Patrick, B., Maxime, C., Imen, C., Mylène, L., & Franck, B. (2021). Performance Characteristics of the Vidas SARS-CoV-2 IgM and IgG Serological Assays. *Journal of Clinical Microbiology*, *59*(4), e02292-20. https://doi.org/10.1128/JCM.02292-20

Naveca, F. G., Nascimento, V., de Souza, V. C., Corado, A. de L., Nascimento, F., Silva, G., Costa, Á., Duarte, D., Pessoa, K., Mejía, M., Brandão, M. J., Jesus, M., Gonçalves, L., da Costa, C. F., Sampaio, V., Barros, D., Silva, M., Mattos, T., Pontes, G., … Bello, G. (2021). COVID-19 in Amazonas, Brazil, was driven by the persistence of endemic lineages and P.1 emergence. *Nature Medicine*, *27*(7), 1230–1238. https://doi.org/10.1038/s41591-021-01378-7

Nelde, A., Bilich, T., Heitmann, J. S., Maringer, Y., Salih, H. R., Roerden, M., Lübke, M., Bauer, J., Rieth, J., Wacker, M., Peter, A., Hörber, S., Traenkle, B., Kaiser, P. D., Rothbauer, U., Becker, M., Junker, D., Krause, G., Strengert, M., … Walz, J. S. (2021). SARS-CoV-2-derived peptides define heterologous and COVID-19-induced T cell recognition. *Nature Immunology*, *22*(1), 74–85. https://doi.org/10.1038/s41590-020-00808-x

O'Toole, Á., Pybus, O. G., Abram, M. E., Kelly, E. J., & Rambaut, A. (2022). Pango lineage designation and assignment using SARS-CoV-2 spike gene nucleotide sequences. *BMC Genomics*, *23*(1), 121. https://doi.org/10.1186/s12864-022-08358-2

Offit, P. A. (2023). Bivalent Covid-19 Vaccines — A Cautionary Tale. *New England Journal of Medicine*, *388*(6), 481–483. https://doi.org/10.1056/NEJMp2215780

Ogata, T., & Tanaka, H. (2023). SARS-CoV-2 Incubation Period during the Omicron BA.5–Dominant Period in Japan. *Emerging Infectious Disease Journal*, *29*(3), 595. https://doi.org/10.3201/eid2903.221360

Okamoto, S., Matsuura, M., Akagi, T., Akashi, M., Tanimoto, T., Ishikawa, T., Takahashi, M., Yamanishi, K., & Mori, Y. (2009). Poly(γ-glutamic acid) nano-particles combined with mucosal influenza virus hemagglutinin vaccine protects against influenza virus infection in mice. *Vaccine*, *27*(42), 5896–5905. https://doi.org/https://doi.org/10.1016/j.vaccine.2009.07.037

Oude Munnink, B. B., Sikkema, R. S., Nieuwenhuijse, D. F., Molenaar, R. J., Munger, E., Molenkamp, R., van der Spek, A., Tolsma, P., Rietveld, A., Brouwer, M., Bouwmeester-Vincken, N., Harders, F., Hakze-van der Honing, R., Wegdam-Blans, M. C. A., Bouwstra, R. J., GeurtsvanKessel, C., van der Eijk, A. A., Velkers, F. C., Smit, L. A. M., … Koopmans, M. P. G. (2021). Transmission of SARS-CoV-2 on mink farms between humans and mink and back to humans. *Science*, *371*(6525), 172–177. https://doi.org/10.1126/science.abe5901

Painter, M. M., Mathew, D., Goel, R. R., Apostolidis, S. A., Pattekar, A., Kuthuru, O., Baxter, A. E., Herati, R. S., Oldridge, D. A., Gouma, S., Hicks, P., Dysinger, S., Lundgreen, K. A., Kuri-Cervantes, L., Adamski, S., Hicks, A., Korte, S., Giles, J. R., Weirick, M. E., … Wherry, E. J. (2021). Rapid induction of antigen-specific CD4+ T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. *Immunity*, *54*(9), 2133-2142.e3. https://doi.org/https://doi.org/10.1016/j.immuni.2021.08.001

Pauly, M. D., Procario, M. C., & Lauring, A. S. (2017). A novel twelve class fluctuation test reveals higher than expected mutation rates for influenza A viruses. *ELife*, *6*, e26437. https://doi.org/10.7554/eLife.26437

Payne, R. P., Longet, S., Austin, J. A., Skelly, D. T., Dejnirattisai, W., Adele, S., Meardon, N., Faustini, S., Al-Taei, S., Moore, S. C., Tipton, T., Hering, L. M., Angyal, A., Brown, R., Nicols, A. R., Gillson, N., Dobson, S. L., Amini, A., Supasa, P., … Zawia, A. A. T. (2021). Immunogenicity of standard and extended dosing intervals of BNT162b2 mRNA vaccine. *Cell*, *184*(23), 5699-5714.e11. https://doi.org/https://doi.org/10.1016/j.cell.2021.10.011

Pekar, J. E., Magee, A., Parker, E., Moshiri, N., Izhikevich, K., Havens, J. L., Gangavarapu, K., Malpica Serrano, L. M., Crits-Christoph, A., Matteson, N. L., Zeller, M., Levy, J. I., Wang, J. C., Hughes, S., Lee, J., Park, H., Park, M.-S., Ching Zi Yan, K., Lin, R. T. P., … Wertheim, J. O. (2022). The molecular epidemiology of multiple zoonotic origins of SARS-CoV-2. *Science*, *377*(6609), 960–966. https://doi.org/10.1126/science.abp8337

Peluso, M. J., Takahashi, S., Hakim, J., Kelly, J. D., Torres, L., Iyer, N. S., Turcios, K., Janson, O., Munter, S. E., Thanh, C., Donatelli, J., Nixon, C. C., Hoh, R., Tai, V., Fehrman, E. A., Hernandez, Y., Spinelli, M. A., Gandhi, M., Palafox, M.-A., … Greenhouse, B. (2023). SARS-CoV-2 antibody magnitude and detectability are driven by disease severity, timing, and assay. *Science Advances*, *7*(31), eabh3409. https://doi.org/10.1126/sciadv.abh3409

Pérez-González, A., Araújo-Ameijeiras, A., Fernández-Villar, A., Crespo, M., Poveda, E., & Cohort COVID-19 of the Galicia Sur Health Research Institute. (2022). Long COVID in hospitalized and non-hospitalized patients in a large cohort in Northwest Spain, a prospective cohort study. *Scientific Reports*, *12*(1), 3369. https://doi.org/10.1038/s41598-022-07414-x

Pickering, S., Batra, R., Merrick, B., Snell, L. B., Nebbia, G., Douthwaite, S., Reid, F., Patel, A., Kia Ik, M. T., Patel, B., Charalampous, T., Alcolea-Medina, A., Lista, M. J., Cliff, P. R., Cunningham, E., Mullen, J., Doores, K. J., Edgeworth, J. D., Malim, M. H., … Galão, R. P. (2021). Comparative performance of SARS-CoV-2 lateral flow antigen tests and association with detection of infectious

virus in clinical specimens: a single-centre laboratory evaluation study. *The Lancet Microbe*, *2*(9), e461–e471. https://doi.org/https://doi.org/10.1016/S2666-5247(21)00143-9

Piec, I., English, E., Thomas, M. A., Dervisevic, S., Fraser, W. D., & John, W. G. (2021). Performance of SARS-CoV-2 serology tests: Are they good enough? *PloS One*, *16*(2), e0245914. https://doi.org/10.1371/journal.pone.0245914

Polack, F. P., Thomas, S. J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J. L., Pérez Marc, G., Moreira, E. D., Zerbini, C., Bailey, R., Swanson, K. A., Roychoudhury, S., Koury, K., Li, P., Kalina, W. V, Cooper, D., Frenck, R. W., Hammitt, L. L., … Gruber, W. C. (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *New England Journal of Medicine*, *383*(27), 2603–2615. https://doi.org/10.1056/NEJMoa2034577

Prendecki, M., Clarke, C., Brown, J., Cox, A., Gleeson, S., Guckian, M., Randell, P., Pria, A. D., Lightstone, L., Xu, X.-N., Barclay, W., McAdoo, S. P., Kelleher, P., & Willicombe, M. (2021). Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. *The Lancet*, *6736*(21), 10–12. https://doi.org/10.1016/S0140-6736(21)00502-X

Prévost, J., Gasser, R., Beaudoin-Bussières, G., Richard, J., Duerr, R., Laumaea, A., Anand, S. P., Goyette, G., Benlarbi, M., Ding, S., Medjahed, H., Lewin, A., Perreault, J., Tremblay, T., Gendron-Lepage, G., Gauthier, N., Carrier, M., Marcoux, D., Piché, A., … Finzi, A. (2020). Cross-Sectional Evaluation of Humoral Responses against SARS-CoV-2 Spike. *Cell Reports Medicine*, *1*(7), 100–126. https://doi.org/10.1016/j.xcrm.2020.100126

Puhach, O., Meyer, B., & Eckerle, I. (2023). SARS-CoV-2 viral load and shedding kinetics. *Nature Reviews Microbiology*, *21*(3), 147–161. https://doi.org/10.1038/s41579-022-00822-w

Pulendran, B., & Ahmed, R. (2011). Immunological mechanisms of vaccination. *Nature Immunology*, *12*(6), 509–517. https://doi.org/10.1038/ni.2039

Qi, H., Liu, B., Wang, X., & Zhang, L. (2022). The humoral response and antibodies against SARS-CoV-2 infection. *Nature Immunology*, *23*(7), 1008–1020. https://doi.org/10.1038/s41590-022-01248-5

Qu, J., Wu, C., Li, X., Zhang, G., Jiang, Z., Li, X., Zhu, Q., & Liu, L. (2020). Profile of Immunoglobulin G and IgM Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clinical Infectious Diseases*, *71*(16), 2255–2258. https://doi.org/10.1093/cid/ciaa489

Rammensee, H. G., Falk, K., & Rötzschke, O. (1993). Peptides Naturally Presented by MHC Class I Molecules. *Annual Review of Immunology*, *11*(1), 213–244. https://doi.org/10.1146/annurev.iy.11.040193.001241

Redondo, N., Zaldívar-López, S., Garrido, J. J., & Montoya, M. (2021). SARS-CoV-2 Accessory Proteins in Viral Pathogenesis: Knowns and Unknowns. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.708264

Regev-Yochay, G., Gonen, T., Gilboa, M., Mandelboim, M., Indenbaum, V., Amit, S., Meltzer, L., Asraf, K., Cohen, C., Fluss, R., Biber, A., Nemet, I., Kliker, L., Joseph, G., Doolman, R., Mendelson, E., Freedman, L. S., Harats, D., Kreiss, Y., & Lustig, Y. (2022). Efficacy of a Fourth Dose of Covid-19 mRNA Vaccine against Omicron. *New England Journal of Medicine*, *386*(14), 1377–1380. https://doi.org/10.1056/NEJMc2202542

Rha, M.-S., Jeong, H. W., Ko, J.-H., Choi, S. J., Seo, I.-H., Lee, J. S., Sa, M., Kim, A. R., Joo, E.-J., Ahn, J. Y., Kim, J. H., Song, K.-H., Kim, E. S., Oh, D. H., Ahn, M. Y., Choi, H. K., Jeon, J. H., Choi, J.-P., Kim, H. Bin, … Shin, E.-C. (2021). PD-1-Expressing SARS-CoV-2-Specific $CD8^+$ T Cells Are Not Exhausted, but Functional in Patients with COVID-19. *Immunity*, *54*(1), 44-52.e3. https://doi.org/10.1016/j.immuni.2020.12.002

Ribeiro, R. M., Li, H., Wang, S., Stoddard, M. B., Learn, G. H., Korber, B. T., Bhattacharya, T., Guedj, J., Parrish, E. H., Hahn, B. H., Shaw, G. M., & Perelson, A. S. (2012). Quantifying the Diversification of Hepatitis C Virus (HCV) during Primary Infection: Estimates of the In Vivo Mutation Rate. *PLOS Pathogens*, *8*(8), 1–13. https://doi.org/10.1371/journal.ppat.1002881

Rogers, T. F., Zhao, F., Huang, D., Beutler, N., Burns, A., He, W., Limbo, O., Smith, C., Song, G., Woehl, J., Yang, L., Abbott, R. K., Callaghan, S., Garcia, E., Hurtado, J., Parren, M., Peng, L., Ramirez, S., Ricketts, J., … Burton, D. R. (2020). Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. *Science*, *369*(6506), 956–963. https://doi.org/10.1126/science.abc7520

Röltgen, K., Nielsen, S. C. A., Silva, O., Younes, S. F., Zaslavsky, M., Costales, C., Yang, F., Wirz, O. F., Solis, D., Hoh, R. A., Wang, A., Arunachalam, P. S., Colburg, D., Zhao, S., Haraguchi, E., Lee, A. S., Shah, M. M., Manohar, M., Chang, I., … Boyd, S. D. (2022). Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. *Cell*, *185*(6), 1025-1040.e14. https://doi.org/https://doi.org/10.1016/j.cell.2022.01.018

Röltgen, K., Powell, A. E., Wirz, O. F., Stevens, B. A., Hogan, C. A., Najeeb, J., Hunter, M., Wang, H., Sahoo, M. K., Huang, C., Yamamoto, F., Manohar, M., Manalac, J., Otrelo-Cardoso, A. R., Pham, T. D., Rustagi, A., Rogers, A. J., Shah, N. H., Blish, C. A., … Boyd, S. D. (2020). Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Science Immunology*, *5*(54), eabe0240. https://doi.org/10.1126/sciimmunol.abe0240

Ruggiero, A., Piubelli, C., Calciano, L., Accordini, S., Valenti, M. T., Carbonare, L. D., Siracusano, G., Temperton, N., Tiberti, N., Longoni, S. S., Pizzato, M., Accordini, S., Fantoni, T., Lopalco, L., Beretta, A., Bisoffi, Z., & Zipeto, D. (2022). SARS-CoV-2 vaccination elicits unconventional IgM specific responses in naive and previously COVID-19-infected individuals. *EBioMedicine*, *77*. https://doi.org/10.1016/j.ebiom.2022.103888

Rusanen, J., Kareinen, L., Szirovicza, L., Uğurlu, H., Levanov, L., Jääskeläinen, A., Ahava, M., Kurkela, S., Saksela, K., Hedman, K., Vapalahti, O., & Hepojoki, J. (2021). A Generic, Scalable, and Rapid Time-Resolved Förster Resonance Energy Transfer-Based Assay for Antigen Detection—SARS-CoV-2 as a Proof of Concept. *MBio*, *12*(3), e00902-21. https://doi.org/10.1128/mBio.00902-21

Russell, C. D., Lone, N. I., & Baillie, J. K. (2023). Comorbidities, multimorbidity and COVID-19. *Nature Medicine*, *29*(2), 334–343. https://doi.org/10.1038/s41591-022-02156-9

Rydyznski Moderbacher, C., Ramirez, S. I., Dan, J. M., Grifoni, A., Hastie, K. M., Weiskopf, D., Belanger, S., Abbott, R. K., Kim, C., Choi, J., Kato, Y., Crotty, E. G., Kim, C., Rawlings, S. A., Mateus, J., Tse, L. P. V., Frazier, A., Baric, R., Peters, B., … Crotty, S. (2020). Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell*, *183*(4), 996-1012.e19. https://doi.org/10.1016/j.cell.2020.09.038

Sahin, U., Muik, A., Vogler, I., Derhovanessian, E., Kranz, L. M., Vormehr, M., Quandt, J., Bidmon, N., Ulges, A., Baum, A., Pascal, K. E., Maurus, D., Brachtendorf, S., Lörks, V., Sikorski, J., Koch, P., Hilker, R., Becker, D., Eller, A.-K., … Türeci, Ö. (2021). BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. *Nature*, *595*(7868), 572–577. https://doi.org/10.1038/s41586-021-03653-6

Sego, T. J., Aponte-Serrano, J. O., Ferrari Gianlupi, J., Heaps, S. R., Breithaupt, K., Brusch, L., Crawshaw, J., Osborne, J. M., Quardokus, E. M., Plemper, R. K., & Glazier, J. A. (2020). A modular framework for multiscale, multicellular, spatiotemporal modeling of acute primary viral infection and immune response in epithelial tissues and its application to drug therapy timing and effectiveness. *PLOS Computational Biology*, *16*(12), e1008451. https://doi.org/10.1371/journal.pcbi.1008451

Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Strålin, K., Gorin, J.-B., Olsson, A., Llewellyn-Lacey, S., Kamal, H., Bogdanovic, G., Muschiol, S., Wullimann, D. J., Kammann, T., Emgård, J., Parrot, T., Folkesson, E., Akber, M., Berglin, L., Bergsten, H., Brighenti, S., … Buggert, M. (2020). Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. *Cell*, *183*(1), 158-168.e14. https://doi.org/10.1016/j.cell.2020.08.017

Sette, A., & Crotty, S. (2021). Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell*, *184*(4), 861–880. https://doi.org/10.1016/j.cell.2021.01.007

Seydoux, E., Homad, L. J., MacCamy, A. J., Parks, K. R., Hurlburt, N. K., Jennewein, M. F., Akins, N. R., Stuart, A. B., Wan, Y.-H., Feng, J., Whaley, R. E., Singh, S., Boeckh, M., Cohen, K. W., McElrath, M. J., Englund, J. A., Chu, H. Y., Pancera, M., McGuire, A. T., & Stamatatos, L. (2020). Analysis of a SARS-CoV-2-Infected Individual Reveals Development of Potent Neutralizing Antibodies with Limited Somatic Mutation. *Immunity*, *53*(1), 98-105.e5. https://doi.org/https://doi.org/10.1016/j.immuni.2020.06.001

Sheikh, A., McMenamin, J., Taylor, B., & Robertson, C. (2021). SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness. *The Lancet*, *397*(10293), 2461–2462. https://doi.org/https://doi.org/10.1016/S0140-6736(21)01358-1

Singh, C., Verma, S., Reddy, P., Diamond, M. S., Curiel, D. T., Patel, C., Jain, M. K., Redkar, S. V., Bhate, A. S., Gundappa, V., Konatham, R., Toppo, L., Joshi, A. C., Kushwaha, J. S., Singh, A. P., Bawankule, S., Ella, R., Prasad, S., Ganneru, B., … Vadrevu, K. M. (2023). Phase III Pivotal comparative clinical trial of intranasal (iNCOVACC) and intramuscular COVID 19 vaccine (Covaxin®). *Npj Vaccines*, *8*(1), 125. https://doi.org/10.1038/s41541-023-00717-8

Skelly, D. T., Harding, A. C., Gilbert-Jaramillo, J., Knight, M. L., Longet, S., Brown, A., Adele, S., Adland, E., Brown, H., Chinnakannan, S., Donnison, T., Ali, M., Rongkard, P., Pace, M., Zacharopoulou, P., Robinson, N., Csala, A., De Lara, C., Hutchings, C. L., … James, W. S. (2021). Two doses of SARS-CoV-2 vaccination induce robust immune responses to emerging SARS-CoV-2 variants of concern. *Nature Communications*, *12*(1), 5061. https://doi.org/10.1038/s41467-021-25167-5

Sokal, A., Barba-Spaeth, G., Fernández, I., Broketa, M., Azzaoui, I., de La Selle, A., Vandenberghe, A., Fourati, S., Roeser, A., Meola, A., Bouvier-Alias, M., Crickx, E., Languille, L., Michel, M., Godeau, B., Gallien, S., Melica, G., Nguyen, Y., Zarrouk, V., … Mahévas, M. (2021). mRNA vaccination of naive and COVID-19-recovered individuals elicits potent memory B cells that recognize SARS-CoV-2 variants. *Immunity*, *54*(12), 2893-2907.e5. https://doi.org/https://doi.org/10.1016/j.immuni.2021.09.011

Solastie, A., Nieminen, T., Ekström, N., Nohynek, H., Lehtonen, L., Palmu, A. A., & Melin, M. (2023). Changes in SARS-CoV-2 seroprevalence and population immunity in Finland, 2020–2022. *Emerging Microbes & Infections*, *12*(2), 1–14. https://doi.org/10.1080/22221751.2023.2222849

Soresina, A., Moratto, D., Chiarini, M., Paolillo, C., Baresi, G., Focà, E., Bezzi, M., Baronio, B., Giacomelli, M., & Badolato, R. (2020). Two X-linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover. *Pediatric Allergy and Immunology*, *31*(5), 565–569. https://doi.org/https://doi.org/10.1111/pai.13263

Stone, S., Rothan, H. A., Natekar, J. P., Kumari, P., Sharma, S., Pathak, H., Arora, K., Auroni, T. T., & Kumar, M. (2022). SARS-CoV-2 Variants of Concern Infect the Respiratory Tract and Induce Inflammatory Response in Wild-Type Laboratory Mice. *Viruses*, *14*(1). https://doi.org/10.3390/v14010027

Su, Y., Chen, D., Yuan, D., Lausted, C., Choi, J., Dai, C. L., Voillet, V., Duvvuri, V. R., Scherler, K., Troisch, P., Baloni, P., Qin, G., Smith, B., Kornilov, S. A., Rostomily, C., Xu, A., Li, J., Dong, S., Rothchild, A., … Heath, J. R. (2020). Multi-Omics Resolves a Sharp Disease-State Shift between Mild and Moderate COVID-19. *Cell*, *183*(6), 1479-1495.e20. https://doi.org/https://doi.org/10.1016/j.cell.2020.10.037

Sun, B., Feng, Y., Mo, X., Zheng, P., Wang, Q., Li, P., Peng, P., Liu, X., Chen, Z., Huang, H., Zhang, F., Luo, W., Niu, X., Hu, P., Wang, L., Peng, H., Huang, Z., Feng, L., Li, F., … Chen, L. (2020). Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. *Emerging Microbes & Infections*, *9*(1), 940–948. https://doi.org/10.1080/22221751.2020.1762515

Supasa, P., Zhou, D., Dejnirattisai, W., Liu, C., Mentzer, A. J., Ginn, H. M., Zhao, Y., Duyvesteyn, H. M. E., Nutalai, R., Tuekprakhon, A., Wang, B., Paesen, G. C., Slon-Campos, J., López-Camacho, C., Hallis, B., Coombes, N., Bewley, K. R., Charlton, S., Walter, T. S., … Screaton, G. R. (2021).

Reduced neutralization of SARS-CoV-2 B.1.1.7 variant by convalescent and vaccine sera. *Cell*, *184*(8), 2201-2211.e7. https://doi.org/10.1016/j.cell.2021.02.033

Suthar, M. S., Zimmerman, M. G., Kauffman, R. C., Mantus, G., Linderman, S. L., Hudson, W. H., Vanderheiden, A., Nyhoff, L., Davis, C. W., Adekunle, O., Affer, M., Sherman, M., Reynolds, S., Verkerke, H. P., Alter, D. N., Guarner, J., Bryksin, J., Horwath, M. C., Arthur, C. M., … Wrammert, J. (2020). Rapid Generation of Neutralizing Antibody Responses in COVID-19 Patients. *Cell Reports Medicine*, *1*(3). https://doi.org/10.1016/j.xcrm.2020.100040

Tada, T., Zhou, H., Samanovic, M. I., Dcosta, B. M., Cornelius, A., Herati, R. S., Mulligan, M. J., & Landau, N. R. (2022). Neutralization of SARS-CoV-2 Variants by mRNA and Adenoviral Vector Vaccine-Elicited Antibodies. *Frontiers in Immunology*, *Mar 8*(13), 797589. https://doi.org/10.3389/fimmu.2022.797589

Tan, A. T., Linster, M., Tan, C. W., Le Bert, N., Chia, W. N., Kunasegaran, K., Zhuang, Y., Tham, C. Y. L., Chia, A., Smith, G. J. D., Young, B., Kalimuddin, S., Low, J. G. H., Lye, D., Wang, L.-F., & Bertoletti, A. (2021). Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. *Cell Reports*, *34*(6), 108728. https://doi.org/https://doi.org/10.1016/j.celrep.2021.108728

Tan, C. W., Chia, W. N., Qin, X., Liu, P., Chen, M. I.-C., Tiu, C., Hu, Z., Chen, V. C.-W., Young, B. E., Sia, W. R., Tan, Y.-J., Foo, R., Yi, Y., Lye, D. C., Anderson, D. E., & Wang, L.-F. (2020). A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. *Nature Biotechnology*, *38*(9), 1073–1078. https://doi.org/10.1038/s41587-020-0631-z

Tang, F., Quan, Y., Xin, Z.-T., Wrammert, J., Ma, M.-J., Lv, H., Wang, T.-B., Yang, H., Richardus, J. H., Liu, W., & Cao, W.-C. (2011). Lack of Peripheral Memory B Cell Responses in Recovered Patients with Severe Acute Respiratory Syndrome: A Six-Year Follow-Up Study. *The Journal of Immunology*, *186*(12), 7264–7268. https://doi.org/10.4049/jimmunol.0903490

Tariq, H., Batool, S., Asif, S., Ali, M., & Abbasi, B. H. (2022). Virus-Like Particles: Revolutionary Platforms for Developing Vaccines Against Emerging Infectious Diseases. *Frontiers in Microbiology*, *12*. https://doi.org/doi.org/10.3389/fmicb.2021.790121

Tarke, A., Coelho, C. H., Zhang, Z., Dan, J. M., Yu, E. D., Methot, N., Bloom, N. I., Goodwin, B., Phillips, E., Mallal, S., Sidney, J., Filaci, G., Weiskopf, D., da Silva Antunes, R., Crotty, S., Grifoni, A., & Sette, A. (2022). SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell*, *185*(5), 847-859.e11. https://doi.org/10.1016/j.cell.2022.01.015

Tarke, A., Sidney, J., Kidd, C. K., Dan, J. M., Ramirez, S. I., Yu, E. D., Mateus, J., da Silva Antunes, R., Moore, E., Rubiro, P., Methot, N., Phillips, E., Mallal, S., Frazier, A., Rawlings, S. A., Greenbaum, J. A., Peters, B., Smith, D. M., Crotty, S., … Sette, A. (2021). Comprehensive analysis of T cell immunodominance and immunoprevalence of SARS-CoV-2 epitopes in COVID-19 cases. *Cell Reports Medicine*, *2*(2). https://doi.org/10.1016/j.xcrm.2021.100204

Tegally, H., Wilkinson, E., Giovanetti, M., Iranzadeh, A., Fonseca, V., Giandhari, J., Doolabh, D., Pillay, S., San, E. J., Msomi, N., Mlisana, K., von Gottberg, A., Walaza, S., Allam, M., Ismail, A., Mohale, T., Glass, A. J., Engelbrecht, S., Van Zyl, G., … de Oliveira, T. (2021). Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*, *592*(7854), 438–443. https://doi.org/10.1038/s41586-021-03402-9

Temmam, S., Vongphayloth, K., Baquero, E., Munier, S., Bonomi, M., Regnault, B., Douangboubpha, B., Karami, Y., Chrétien, D., Sanamxay, D., Xayaphet, V., Paphaphanh, P., Lacoste, V., Somlor, S., Lakeomany, K., Phommavanh, N., Pérot, P., Dehan, O., Amara, F., … Eloit, M. (2022). Bat coronaviruses related to SARS-CoV-2 and infectious for human cells. *Nature*, *604*(7905), 330–336. https://doi.org/10.1038/s41586-022-04532-4

Tenforde, M. W., Self, W. H., Gaglani, M., Ginde, A. A., Douin, D. J., & Talbot, H. K. (2022). Effectiveness of mRNA Vaccination in Preventing COVID-19 – Associated Invasive Mechanical

Ventilation and Death — United States , March 2021 – January 2022. *MMWR Morb Mortal Wkly Rep*, *71*(12), 459–465.

Terreri, S., Piano Mortari, E., Vinci, M. R., Russo, C., Alteri, C., Albano, C., Colavita, F., Gramigna, G., Agrati, C., Linardos, G., Coltella, L., Colagrossi, L., Deriu, G., Ciofi Degli Atti, M., Rizzo, C., Scarsella, M., Brugaletta, R., Camisa, V., Santoro, A., … Carsetti, R. (2022). Persistent B cell memory after SARS-CoV-2 vaccination is functional during breakthrough infections. *Cell Host & Microbe*, *30*(3), 400-408.e4. https://doi.org/https://doi.org/10.1016/j.chom.2022.01.003

Thanh Le, T., Andreadakis, Z., Kumar, A., Gómez Román, R., Tollefsen, S., Saville, M., & Mayhew, S. (2020). The COVID-19 vaccine development landscape. *Nature Reviews. Drug Discovery*, *19*(5), 305–306. https://doi.org/10.1038/d41573-020-00073-5

Travieso, T., Li, J., Mahesh, S., Mello, J. D. F. R. E., & Blasi, M. (2022). The use of viral vectors in vaccine development. *Npj Vaccines*, *7*(1), 75. https://doi.org/10.1038/s41541-022-00503-y

Trolle, T., McMurtrey, C. P., Sidney, J., Bardet, W., Osborn, S. C., Kaever, T., Sette, A., Hildebrand, W. H., Nielsen, M., & Peters, B. (2016). The Length Distribution of Class I-Restricted T Cell Epitopes Is Determined by Both Peptide Supply and MHC Allele-Specific Binding Preference. *Journal of Immunology*, *196*(4), 1480–1487. https://doi.org/10.4049/jimmunol.1501721

Tsang, T. K., Cauchemez, S., Perera, R. A. P. M., Freeman, G., Fang, V. J., Ip, D. K. M., Leung, G. M., Malik Peiris, J. S., & Cowling, B. J. (2014). Association Between Antibody Titers and Protection Against Influenza Virus Infection Within Households. *The Journal of Infectious Diseases*, *210*(5), 684–692. https://doi.org/10.1093/infdis/jiu186

Tseng, C.-T., Sbrana, E., Iwata-Yoshikawa, N., Newman, P. C., Garron, T., Atmar, R. L., Peters, C. J., & Couch, R. B. (2012). Immunization with SARS Coronavirus Vaccines Leads to Pulmonary Immunopathology on Challenge with the SARS Virus. *PLOS ONE*, *7*(4), 1–13. https://doi.org/10.1371/journal.pone.0035421

Tso, F. Y., Lidenge, S. J., Peña, P. B., Clegg, A. A., Ngowi, J. R., Mwaiselage, J., Ngalamika, O., Julius, P., West, J. T., & Wood, C. (2021). High prevalence of pre-existing serological cross-reactivity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) in sub-Saharan Africa. *International Journal of Infectious Diseases*, *102*, 577–583. https://doi.org/https://doi.org/10.1016/j.ijid.2020.10.104

V'kovski, P., Kratzel, A., Steiner, S., Stalder, H., & Thiel, V. (2021). Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews Microbiology*, *19*(3), 155–170. https://doi.org/10.1038/s41579-020-00468-6

van der Hoek, L., Pyrc, K., Jebbink, M. F., Vermeulen-Oost, W., Berkhout, R. J. M., Wolthers, K. C., Wertheim-van Dillen, P. M. E., Kaandorp, J., Spaargaren, J., & Berkhout, B. (2004). Identification of a new human coronavirus. *Nature Medicine*, *10*(4), 368–373. https://doi.org/10.1038/nm1024

Van Elslande, J., Decru, B., Jonckheere, S., Van Wijngaerden, E., Houben, E., Vandecandelaere, P., Indevuyst, C., Depypere, M., Desmet, S., André, E., Van Ranst, M., Lagrou, K., & Vermeersch, P. (2020). Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by four automated immunoassays and three ELISAs. *Clinical Microbiology and Infection*, *26*(11), 1557.e1-1557.e7. https://doi.org/https://doi.org/10.1016/j.cmi.2020.07.038

Vogels, C. B. F., Brito, A. F., Wyllie, A. L., Fauver, J. R., Ott, I. M., Kalinich, C. C., Petrone, M. E., Casanovas-Massana, A., Catherine Muenker, M., Moore, A. J., Klein, J., Lu, P., Lu-Culligan, A., Jiang, X., Kim, D. J., Kudo, E., Mao, T., Moriyama, M., Oh, J. E., … Grubaugh, N. D. (2020). Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. *Nature Microbiology*, *5*(10), 1299–1305. https://doi.org/10.1038/s41564-020-0761-6

Walsh, E. E., Frenck, R. W., Falsey, A. R., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Mulligan, M. J., Bailey, R., Swanson, K. A., Li, P., Koury, K., Kalina, W., Cooper, D., Fontes-Garfias, C., Shi, P.-Y., Türeci, Ö., Tompkins, K. R., … Gruber, W. C. (2020). Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *New England Journal of Medicine*, *383*(25), 2439–2450. https://doi.org/10.1056/nejmoa2027906

Wang, H., Yuan, Y., Xiao, M., Chen, L., Zhao, Y., Zhang, H., Long, P., Zhou, Y., Xu, X., Lei, Y., Wu, B., Diao, T., Cai, H., Liu, L., Shao, Z., Wang, J., Bai, Y., Wang, K., Peng, M., … Wu, T. (2021). Dynamics of the SARS-CoV-2 antibody response up to 10 months after infection. *Cellular & Molecular Immunology*, *18*(7), 1832–1834. https://doi.org/10.1038/s41423-021-00708-6

Wang, Z., Lorenzi, J. C. C., Muecksch, F., Finkin, S., Viant, C., Gaebler, C., Cipolla, M., Hoffmann, H.-H., Oliveira, T. Y., Oren, D. A., Ramos, V., Nogueira, L., Michailidis, E., Robbiani, D. F., Gazumyan, A., Rice, C. M., Hatziioannou, T., Bieniasz, P. D., Caskey, M., & Nussenzweig, M. C. (2021). Enhanced SARS-CoV-2 neutralization by dimeric IgA. *Science Translational Medicine*, *13*(577), eabf1555. https://doi.org/10.1126/scitranslmed.abf1555

Wang, Z., Muecksch, F., Schaefer-Babajew, D., Finkin, S., Viant, C., Gaebler, C., Hoffmann, H.-H., Barnes, C. O., Cipolla, M., Ramos, V., Oliveira, T. Y., Cho, A., Schmidt, F., Da Silva, J., Bednarski, E., Aguado, L., Yee, J., Daga, M., Turroja, M., … Nussenzweig, M. C. (2021). Naturally enhanced neutralizing breadth against SARS-CoV-2 one year after infection. *Nature*, *595*(7867), 426–431. https://doi.org/10.1038/s41586-021-03696-9

Ward, H., Whitaker, M., Flower, B., Tang, S. N., Atchison, C., Darzi, A., Donnelly, C. A., Cann, A., Diggle, P. J., Ashby, D., Riley, S., Barclay, W. S., Elliott, P., & Cooke, G. S. (2022). Population antibody responses following COVID-19 vaccination in 212,102 individuals. *Nature Communications*, *13*(1), 907. https://doi.org/10.1038/s41467-022-28527-x

Wei, J., Matthews, P. C., Stoesser, N., Diamond, I., Studley, R., Rourke, E., Cook, D., Bell, J. I., Newton, J. N., Farrar, J., Howarth, A., Marsden, B. D., Hoosdally, S., Jones, E. Y., Stuart, D. I., Crook, D. W., Peto, T. E. A., Walker, A. S., Eyre, D. W., … The COVID-19 Infection Survey team. (2022). SARS-CoV-2 antibody trajectories after a single COVID-19 vaccination with and without prior infection. *Nature Communications*, *13*(1), 3748. https://doi.org/10.1038/s41467-022-31495-x

Wei, J., Matthews, P. C., Stoesser, N., Maddox, T., Lorenzi, L., Studley, R., Bell, J. I., Newton, J. N., Farrar, J., Diamond, I., Rourke, E., Howarth, A., Marsden, B. D., Hoosdally, S., Jones, E. Y., Stuart, D. I., Crook, D. W., Peto, T. E. A., Pouwels, K. B., … The COVID-19 Infection Survey team. (2021). Anti-spike antibody response to natural SARS-CoV-2 infection in the general population. *Nature Communications*, *12*(1), 6250. https://doi.org/10.1038/s41467-021-26479-2

Weiskopf, D., Schmitz, K. S., Raadsen, M. P., Grifoni, A., Okba, N. M. A., Endeman, H., van den Akker, J. P. C., Molenkamp, R., Koopmans, M. P. G., van Gorp, E. C. M., Haagmans, B. L., de Swart, R. L., Sette, A., & de Vries, R. D. (2020). Phenotype and kinetics of SARS-CoV-2–specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Science Immunology*, *5*(48), eabd2071. https://doi.org/10.1126/sciimmunol.abd2071

Wells, D. A., Cantoni, D., Mayora-Neto, M., Genova, C. Di, Sampson, A., Ferrari, M., Carnell, G., Nadesalingam, A., Smith, P., Chan, A., Raddi, G., Castillo-Olivares, J., Baxendale, H., Temperton, N., & Heeney, J. L. (2022). Human seasonal coronavirus neutralization and COVID-19 severity. *Journal of Medical Virology*, *94*(10), 4820–4829. https://doi.org/https://doi.org/10.1002/jmv.27937

Wheatley, A. K., Fox, A., Tan, H.-X., Juno, J. A., Davenport, M. P., Subbarao, K., & Kent, S. J. (2021). Immune imprinting and SARS-CoV-2 vaccine design. *Trends in Immunology*, *42*(11), 956–959. https://doi.org/10.1016/j.it.2021.09.001

Wheatley, A. K., Juno, J. A., Wang, J. J., Selva, K. J., Reynaldi, A., Tan, H.-X., Lee, W. S., Wragg, K. M., Kelly, H. G., Esterbauer, R., Davis, S. K., Kent, H. E., Mordant, F. L., Schlub, T. E., Gordon, D. L., Khoury, D. S., Subbarao, K., Cromer, D., Gordon, T. P., … Kent, S. J. (2021). Evolution of immune responses to SARS-CoV-2 in mild-moderate COVID-19. *Nature Communications*, *12*(1), 1162. https://doi.org/10.1038/s41467-021-21444-5

WHO. (2019). *MERS-CoV Global Summary and Assessment of Risk*. https://www.who.int/publications/i/item/WHO-MERS-RA-2022.1

WHO. (2020). *Diagnostic testing for SARS-CoV-2 Interim guidance 11 September 2020*. https://doi.org/10.1503/cmaj.200858

WHO. (2021). *Vaccine efficacy, effectiveness and protection*. https://www.who.int/news-room/feature-stories/detail/vaccine-efficacy-effectiveness-and-protection

WHO. (2023a). *Status of COVID-19 Vaccines within WHO EUL / PQ evaluation process*. https://www.who.int/teams/regulation-prequalification/eul/covid-19

WHO. (2023b). *WHO Coronavirus (COVID-19) Dashboard*. https://covid19.who.int/

Wibmer, C. K., Ayres, F., Hermanus, T., Madzivhandila, M., Kgagudi, P., Oosthuysen, B., Lambson, B. E., de Oliveira, T., Vermeulen, M., van der Berg, K., Rossouw, T., Boswell, M., Ueckermann, V., Meiring, S., von Gottberg, A., Cohen, C., Morris, L., Bhiman, J. N., & Moore, P. L. (2021). SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nature Medicine*. https://doi.org/10.1038/s41591-021-01285-x

Wirsching, S., Harder, L., Heymanns, M., Gröndahl, B., Hilbert, K., Kowalzik, F., Meyer, C., & Gehring, S. (2022). Long-Term, CD4+ Memory T Cell Response to SARS-CoV-2. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.800070

Wolter, N., Jassat, W., Walaza, S., Welch, R., Moultrie, H., Groome, M., Amoako, D. G., Everatt, J., Bhiman, J. N., Scheepers, C., Tebeila, N., Chiwandire, N., du Plessis, M., Govender, N., Ismail, A., Glass, A., Mlisana, K., Stevens, W., Treurnicht, F. K., … Cohen, C. (2022). Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study. *Lancet*, *399*(10323), 437–446. https://doi.org/10.1016/S0140-6736(22)00017-4

Woo, P. C. Y., de Groot, R. J., Haagmans, B., Lau, S. K. P., Neuman, B. W., Perlman, S., Sola, I., van der Hoek, L., Wong, A. C. P., & Yeh, S.-H. (2023). ICTV Virus Taxonomy Profile: Coronaviridae 2023. *Journal of General Virology*, *104*(4). https://doi.org/https://doi.org/10.1099/jgv.0.001843

Woo, P. C. Y., Lau, S. K. P., Chu, C., Chan, K., Tsoi, H., Huang, Y., Wong, B. H. L., Poon, R. W. S., Cai, J. J., Luk, W., Poon, L. L. M., Wong, S. S. Y., Guan, Y., Peiris, J. S. M., & Yuen, K. (2005). *Characterization and Complete Genome Sequence of a Novel Coronavirus , Coronavirus HKU1 , from Patients with Pneumonia*. *79*(2), 884–895. https://doi.org/10.1128/JVI.79.2.884

Worobey, M., Levy, J. I., Malpica Serrano, L., Crits-Christoph, A., Pekar, J. E., Goldstein, S. A., Rasmussen, A. L., Kraemer, M. U. G., Newman, C., Koopmans, M. P. G., Suchard, M. A., Wertheim, J. O., Lemey, P., Robertson, D. L., Garry, R. F., Holmes, E. C., Rambaut, A., & Andersen, K. G. (2022). The Huanan Seafood Wholesale Market in Wuhan was the early epicenter of the COVID-19 pandemic. *Science*, *377*(6609), 951–959. https://doi.org/10.1126/science.abp8715

Wu, C.-H., Chen, P.-J., & Yeh, S.-H. (2014). Nucleocapsid Phosphorylation and RNA Helicase DDX1 Recruitment Enables Coronavirus Transition from Discontinuous to Continuous Transcription. *Cell Host & Microbe*, *16*(4), 462–472. https://doi.org/10.1016/j.chom.2014.09.009

Wu, C., Yin, W., Jiang, Y., & Xu, H. E. (2022). Structure genomics of SARS-CoV-2 and its Omicron variant: drug design templates for COVID-19. *Acta Pharmacologica Sinica*, *43*(12), 3021–3033. https://doi.org/10.1038/s41401-021-00851-w

Wu, F., Zhao, S., Yu, B., Chen, Y.-M., Wang, W., Song, Z.-G., Hu, Y., Tao, Z.-W., Tian, J.-H., Pei, Y.-Y., Yuan, M.-L., Zhang, Y.-L., Dai, F.-H., Liu, Y., Wang, Q.-M., Zheng, J.-J., Xu, L., Holmes, E. C., & Zhang, Y.-Z. (2020). A new coronavirus associated with human respiratory disease in China. *Nature*, *579*(7798), 265–269. https://doi.org/10.1038/s41586-020-2008-3

Wu, Y., Kang, L., Guo, Z., Liu, J., Liu, M., & Liang, W. (2022). Incubation Period of COVID-19 Caused by Unique SARS-CoV-2 Strains: A Systematic Review and Meta-analysis. *JAMA Network Open*, *5*(8), e2228008–e2228008. https://doi.org/10.1001/jamanetworkopen.2022.28008

Yu, H., Liu, B., Zhang, Y., Gao, X., Wang, Q., Xiang, H., Peng, X., Xie, C., Wang, Y., Hu, P., Shi, J., Shi, Q., Zheng, P., Feng, C., Tang, G., Liu, X., Guo, L., Lin, X., Li, J., … Tang, X. (2023). Somatically hypermutated antibodies isolated from SARS-CoV-2 Delta infected patients cross-neutralize heterologous variants. *Nature Communications*, *14*(1), 1058. https://doi.org/10.1038/s41467-023-36761-0

Yu, S., Zhang, P., Liao, M., Zhang, J., Luo, S., Zhai, J., Zhang, Y., Lin, J., Yuan, J., Zhang, Z., Wang, F., & Wei, L. (2022). Detection of SARS-CoV-2 Specific Antibodies in Saliva Samples. *Frontiers in Immunology*, *13*, 880154. https://doi.org/10.3389/fimmu.2022.880154

Zenarruzabeitia, O., Astarloa-Pando, G., Terrén, I., Orrantia, A., Pérez-Garay, R., Seijas-Betolaza, I., Nieto-Arana, J., Imaz-Ayo, N., Pérez-Fernández, S., Arana-Arri, E., & Borrego, F. (2021). T Cell Activation, Highly Armed Cytotoxic Cells and a Shift in Monocytes CD300 Receptors Expression Is Characteristic of Patients With Severe COVID-19. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.655934

Zhang, Q., Xiang, R., Huo, S., Zhou, Y., Jiang, S., Wang, Q., & Yu, F. (2021). Molecular mechanism of interaction between SARS-CoV-2 and host cells and interventional therapy. *Signal Transduction and Targeted Therapy*, *6*(1), 233. https://doi.org/10.1038/s41392-021-00653-w

Zhang, Z., Nomura, N., Muramoto, Y., Ekimoto, T., Uemura, T., Liu, K., Yui, M., Kono, N., Aoki, J., Ikeguchi, M., Noda, T., Iwata, S., Ohto, U., & Shimizu, T. (2022). Structure of SARS-CoV-2 membrane protein essential for virus assembly. *Nature Communications*, *13*(1), 4399. https://doi.org/10.1038/s41467-022-32019-3

Zhao, J., Zhao, J., Mangalam, A. K., Channappanavar, R., Fett, C., Meyerholz, D. K., Agnihothram, S., Baric, R. S., David, C. S., & Perlman, S. (2016). Airway Memory CD4+ T Cells Mediate Protective Immunity against Emerging Respiratory Coronaviruses. *Immunity*, *44*(6), 1379–1391. https://doi.org/https://doi.org/10.1016/j.immuni.2016.05.006

Zhao, S., Peng, Z., & Wang, M. H. (2022). A tentative assessment of the changes in transmissibility and fatality risk associated with Beta SARS-CoV-2 variants in South Africa: an ecological study. *Pathogens and Global Health*, *116*(3), 137–139. https://doi.org/10.1080/20477724.2021.2014236

Zhao, Z., Li, H., Wu, X., Zhong, Y., Zhang, K., Zhang, Y.-P., Boerwinkle, E., & Fu, Y.-X. (2004). Moderate mutation rate in the SARS coronavirus genome and its implications. *BMC Evolutionary Biology*, *4*(1), 21. https://doi.org/10.1186/1471-2148-4-21

Zhou, P., Yang, X. Lou, Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Jiang, R. Di, Liu, M. Q., Chen, Y., Shen, X. R., Wang, X., … Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, *579*(7798), 270–273. https://doi.org/10.1038/s41586-020-2012-7

Zhou, S., Lv, P., Li, M., Chen, Z., Xin, H., Reilly, S., & Zhang, X. (2023). SARS-CoV-2 E protein: Pathogenesis and potential therapeutic development. *Biomedicine & Pharmacotherapy*, *159*, 114242. https://doi.org/10.1016/j.biopha.2023.114242

Zuo, J., Dowell, A. C., Pearce, H., Verma, K., Long, H. M., Begum, J., Aiano, F., Amin-Chowdhury, Z., Hoschler, K., Brooks, T., Taylor, S., Hewson, J., Hallis, B., Stapley, L., Borrow, R., Linley, E., Ahmad, S., Parker, B., Horsley, A., … Moss, P. (2021). Robust SARS-CoV-2-specific T cell immunity is maintained at 6 months following primary infection. *Nature Immunology*, *22*(5), 620–626. https://doi.org/10.1038/s41590-021-00902-8

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU

Painosalama, Turku, Finland 2023

ISBN 978-951-29-9464-9 (PRINT)
ISBN 978-951-29-9465-6 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)