Thesis Title

Immune responsiveness following diverse SARS-CoV-2 vaccination platforms

Thesis submitted for the degree of Doctor of Philosophy in Clinical Medicine
Michaelmas Term 2025

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Reuben College

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Abstract

Even among immunocompetent individuals, immune responses following SARS-CoV-2 vaccination are heterogenous, and the mechanisms underlying this variability are not fully understood. The overall aim of this thesis was to characterise vaccine-induced immune responses in real-world populations and to identify immunological and demographic factors associated with variability in vaccine-induced immune responses.

First, immune responses were characterised in vaccinated UK healthcare workers who self-reported no documented breakthrough infection. These individuals had robust spike-specific antibodies and T-cell responses consistent with vaccination. Approximately half also had non-spike-specific immune responses, suggesting previously unrecognised viral exposure.

Second, blood proteomic profiling of healthy UK vaccine recipients was performed to identify protein markers associated with T-cell responses following mRNA vaccination. Multivariable analysis identified eukaryotic translation initiation factor 5A (EIF5A), interleukin 1 receptor-like 2 (IL1RL2), collagen type IX alpha 1 chain (COL9A1), matrix metallopeptidase 1 (MMP1) and Src kinase-associated phosphoprotein 2 (SKAP2), along with age, sex and serostatus to CMV, as predictors of T-cell responses. Immunophenotypic analysis using high-dimensional flow cytometry further demonstrated that T-cell non-response group exhibited markers associated with T-cell senescence and functional

exhaustion, whereas T-cell response group showed higher frequencies of naïve $CD8^+$ T-cells and regulatory T cells than non-response group.

Third, humoral and cellular immune responses were compared across vaccine platforms before vaccination and one month after the second vaccine dose. mRNA vaccines elicited higher spike-specific responses following vaccination compared with other vaccine platforms in previously infected individuals. Inactivated vaccines induced nucleocapsid-specific IgA responses in blood.

Collectively, this thesis demonstrates that humoral and cellular immune responses to SARS-CoV-2 vaccination are shaped by baseline immune competence, exposure history, and vaccine platform. This work highlights the importance of considering both host immune landscape and vaccine platform to understand vaccine immunogenicity to inform the optimisation of vaccination strategies.

Acknowledgement

I would like to express my sincere gratitude to my supervisors for their guidance and support throughout my DPhil. I am thankful for the opportunities to learn, to develop as a researcher, and to present my work in a range of academic settings. I am also deeply appreciative of their efforts in liaising with funders, collaborators, and institutional partners, and for navigating the administrative and organisational complexities that made this work possible.

To the members of Dunachie laboratory and collaborators in the UK, OUCRU Vietnam, and Indonesia, thank you for your collaboration and support throughout this work, both inside and outside the laboratory. I am deeply grateful for the time, expertise, and resources you shared, and for working alongside me to make this research possible. Special acknowledgement to Melissa Govander (MSD antibody work), Clement Twumasi (proteomic data analysis), and Oliver Burton (immunophenotyping work).

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To my friends in Oxford and around the world, thank you for your prayers, love and comfort. Thank you for checking in and for sharing your passion for science, nature, travel, culture and equality.

To my family and my partner, my constant companions throughout this journey and in life, my safe place, thank you for the countless ways you have taken care of me. Thank you for encouraging me to always be the best version of myself and for loving me regardless. You are the voice of reason and compassion that reminds me of what truly matters.

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Contributions to the work

Chapter 3

The work in this chapter was conducted using stored plasma, serum, SAM strips, PBMCs collected in the PITCH, VIBRANT and COCO studies. Antibody data, including serum IgG and nasal antibodies, were generated by Melissa Govender in Miles Carroll's Lab, University of Oxford, with my assistance with experimental work. The remaining experimental work, including ELISpot assays and proliferation assays, and data analyses were performed by me.

Chapter 4

The work in this chapter was conducted using stored plasma, serum and PBMCs collected in the PITCH, COCO, "Human immune responses to acute virus infections", Newcastle (ref: 20/SC/023), Observational Biobanking study STHObs, and COV-AD studies. Proteomics data were generated by the Multiomics Technology Platforms team at the Centre for Human Genetics, University of Oxford. Proteomics data analyses, including PCA, predictive modelling and immunocompetence score, were performed jointly with Clement Twumasi, University of Oxford. Immunophenotyping data were generated by Oliver Burton in Adrian Liston's Lab, University of Cambridge. The remaining experimental work, including anti-CMV and sandwich ELISAs, and data analyses, including heatmap generation, GO analysis, ELISA data analysis, and flow cytometry data analysis, were performed by me.

Chapter 5

The work in this chapter was conducted using stored serum and PBMCs collected in the INVITE study. Samples and demographic data were collected by Prof. Erni Nelwan, Dr. Suwarti and OUCRU Indonesia research support staff, who also coordinated ethical approvals and the associated material transfer agreement. Antibody data, including IgG, IgA and ACE2 binding antibodies, were generated by Melissa Govender in Miles Carroll's Lab, University of Oxford, with my assistance with experimental work. Colleagues in the Dunachie laboratory, Dr. Srija Moulik and Priyanka Abraham assisted with thawing and plating some of the PBMCs for ELISpot experiments. The remaining experimental work, including ELISpot, ECS and ICS, and data analyses, were performed by me.

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4PL	four-parameter logistic
ACE2	angiotensin-converting enzyme 2
Ad5	adenovirus type 5
ADCC	antibody-dependent-cellular cytotoxicity
ADCD	antibody-dependent complement deposition
ADMP	antibody-dependent monocyte phagocytosis
ADNP	antibody-dependent neutrophil phagocytosis
AH	aluminium hydroxide
AIM	activation-induced marker
AIRE	autoimmune regulator
APC	antigen-presenting cell
APS-1	autoimmune polyendocrine syndrome type-1
BAL	bronchoalveolar lavage
BCG	Bacille Calmette-Guérin
BCR	B-cell receptor
BNT	BioNTech
CD	cluster of differentiation
CD40L	CD40 ligand
CEF	cytomegalovirus (CMV), Epstein-Barr virus (EBV), and influenza virus
cGAS	cyclic GMP-AMP synthase
CMV	cytomegalovirus
COCO	COVID-19 Convalescent immunity
COL9A1	Human collagen type IX alpha 1
ConA	Concanavalin A
COVID-19	coronavirus disease of 2019
CpG-alum	CpG1018 formulated in alum (aluminium hydroxide)
DI	Documented Infection
DN	double negative ($CD4^-CD8^-$)
DTP vaccine	diphtheria, tetanus and pertussis vaccine
dsRNA	double-stranded RNA
E	envelope
EBV	Epstein-Barr virus
EC	ethics committee
ECM	extracellular matrix
EF	extrafollicular
eIF5A	eukaryotic translation initiation factor 5A-1
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
ER	endoplasmic reticulum
ERGIC	ER-to-Golgi intermediate compartment
EVAIC	evaluation of adaptive immunity following inactivated whole-virus vaccination in Indonesian populations
FcR	Fc receptor
FDR	false discovery rate

GC	germinal centre
HCoV	human coronavirus
HCW	healthcare worker
ICOS	inducible T-cell co-stimulator
ICU	intensive care unit
Ig	immunoglobulin
IFN	interferon
IFNAR	Interferon alpha receptor
IFN-γ	interferon-gamma
IFNLR	interferon lambda receptor
IL	interleukin
IL1R	IL1 receptor
IL1RL2	IL1 receptor-like 2
INVITE	real-world assessment of the immunogenicity and effectiveness of COVID-19 vaccines in Indonesia
IQR	interquartile range
IRF	interferon regulatory factors
ISG	IFN-stimulated genes
JAK/STAT	Janus kinase/signal transducers and activators of transcription
LASSO	least absolute shrinkage and selection operator
LN2	liquid nitrogen
M	membrane
MAIT	mucosal-associated invariant T
MAPK	mitogen activated protein kinases
MDA5	melanoma differentiation-associated gene 5
MERS-CoV	Middle East respiratory syndrome coronavirus
MHC	major histocompatibility complex
MMP1	matrix metalloproteinase 1
MoH	ministry of health
MPL	monophosphoryl lipid A
MSD	MesoScale Discovery
MTA	material transfer agreement
N	nucleocapsid
NAb	neutralising antibody
NK	natural killer
NF-κB	nuclear factor-κB
NKI	No Known Infection
NOD2	nucleotide-binding oligomerization domain-containing protein 2
NSP	non-structural protein
NTD	N-terminal domain
OAS1	oligoadenylate synthetase 1
ORF	open reading frame
OxTREC	Oxford Tropical Research Ethics Committee
PAMP	pathogen-associated molecular patterns
PBMC	peripheral blood mononuclear cell
PD-1	programmed cell death protein 1
pDC	plasmacytoid dendritic cell
PITCH	Protective Immunity from T-cells to Covid-19 in Health workers

PRR	pattern recognition receptors
PSO	post-symptom onset
RBD	receptor binding domain
RdRp	RNA-dependent RNA polymerase
REC	research ethics committee
RIG-I	retinoic acid-inducible gene I
RNA	ribonucleic acid
RNase L	ribonuclease L
RTC	replication-transcription complex
S	spike
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SIREN	SARS-CoV-2 immunity and reinfection evaluation
SKAP2	Src Kinase Associated Phosphoprotein 2
ssRNA	single-stranded RNA
STING	stimulator of interferon genes
Tcm	central memory T-cells
TCF	T-cell factor
TCR	T-cell receptor
Tem	effector memory T-cells
Temra	terminally differentiated effector memory T-cells
Tfh	T follicular helper
TGF	transforming growth factor
Th	T helper
Tim-3	T-cell immunoglobulin and mucin domain containing protein 3
TLR	Toll-like receptors
TMPRSS2	transmembrane protease, serine 2
TNF	tumour necrosis factor
Treg	regulatory T-cells
UE	unexposed
UK	United Kingdom
VIBRANT	Vaccine Immunity, Breakthrough & Reinfection - Antibodies & T-cells

1 Introduction

1.1 SARS-CoV-2

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded ribonucleic acid (RNA) virus that belongs to the family *Coronaviridae*. SARS-CoV-2 was first reported in Wuhan, China in December 2019 and causes coronavirus disease 2019 (COVID-19) (N. Zhu et al., 2020). COVID-19 was later declared a pandemic by the World Health Organization (WHO) on 11 March 2020 (World Health Organization, 2020), which lasted for over three years until May 2023 (Lennaro, 2023). By February 2023, there had been 680 million cases documented, and 6.8 million deaths reported worldwide (Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU), 2023). This number is likely underestimated due to variability in screening and reporting across countries.

SARS-CoV-2 can be transmitted from person to person through respiratory droplets via the upper respiratory tract. Once SARS-CoV-2 is inhaled, the virus uses the spike (S) protein on its surface to bind to the host receptor angiotensin-converting enzyme 2 (ACE2) (Walls et al., 2020; Zhou et al., 2020), found mainly on respiratory epithelial cells, and other body tissues including intestine, blood vessels, adipose tissue and heart. The spike protein has two subunits, S1 and S2. The S1 contains the receptor binding domain (RBD) (Tortorici & Veesler, 2019), which binds to the host ACE2 receptor, and an N-terminal domain (NTD). Following RBD-ACE2 binding, the virus uses the S2 subunit to fuse to the host cell membrane with the help of the transmembrane protease, serine 2 (TMPRSS2) (Walls et al., 2020) and the cysteine proteases cathepsin B and L (Hoffmann et al., 2020).

Inside the host cytosol, the virus genome is released and translated by host ribosomes. At the 5’ end, open reading frames (ORF)1a and ORF1b are translated and processed into 15-16 non-structural proteins (NSP) which include viral replication-transcription complex (RTC) (V'Kovski et al., 2021) (Figure 1-1). At the 3’ end, ORFs of structural and accessory proteins are translated and transported through the host endoplasmic reticulum (ER) and Golgi apparatus. In the ER-to-Golgi intermediate compartment (ERGIC), RNA interacts with nucleocapsid (N) proteins and buds in the secretory vesicles. New virus particles are then released by exocytosis to infect other cells.

The average incubation time for the ancestral SARS-CoV-2 strain was approximately 5 days (Li et al., 2020). Common symptoms of COVID-19 include fever, cough, myalgia and diarrhoea (Huang et al., 2020) with 3% of people during the first wave developing severe disease requiring hospitalisation (Salje et al., 2020). Hospitalised patients frequently presented with pneumonia and complications such as acute hypoxaemic respiratory failure and acute cardiac injury (Grasselli et al., 2020; Huang et al., 2020). Risk factors for severe SARS-CoV-2 and mortality include older age, male sex, and the presence of comorbidities associated with immune dysregulation or immune suppression, such as diabetes, chronic kidney disease, cardiovascular diseases, autoimmune disorders, malignancy and secondary immunodeficiencies (Grasselli et al., 2020; Li et al., 2020; Salje et al., 2020; Williamson et al., 2020; Wu et al., 2020).

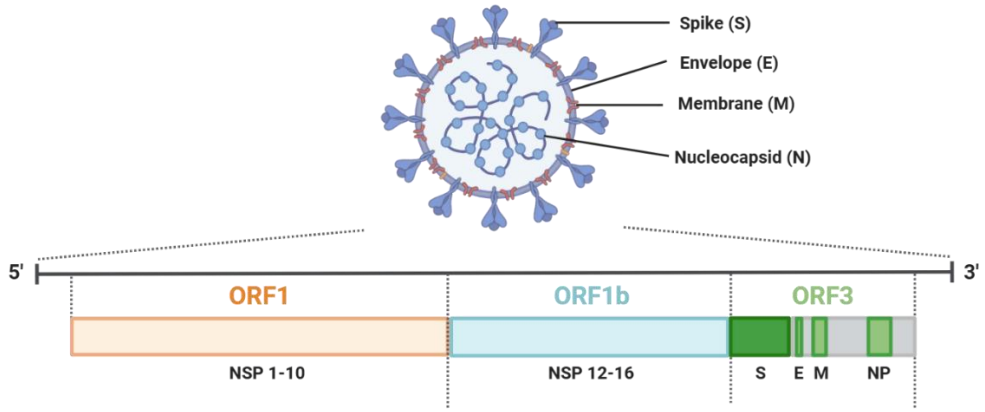

Figure 1-1 Schematic diagram of the SARS-CoV-2 genome

SARS-CoV-2 is a single-stranded RNA virus. The viral RNA is packed by nucleocapsid (N) protein inside the envelope (E) that is studded with spike (S) glycoproteins. At the 5' UTR, ORF1a encodes non-structural protein (NSP)1-10 and ORF1b encodes NSP12-16. At the 3' end, genes encode the structural proteins, such as S, E, M and N. Genes encoding accessory proteins are interspersed among the structural protein genes. *Created with Biorender.com.*

As SARS-CoV-2 spread globally during the pandemic, multiple viral variants emerged through the accumulation of mutations in the viral genome. The WHO classified certain SARS-CoV-2 lineages as variants of concern (VOC) based on evidence of increased transmissibility, immune evasion, and/or reduced responsiveness to existing therapeutics and vaccines (WHO Technical Advisory Group on Virus Evolution (TAG-VE), 2023).

The Alpha variant (B.1.1.7) was retrospectively estimated to have emerged in September 2020 and was first detected and formally reported in the United Kingdom in December 2020 (Public Health England, 2020). Alpha carries mutations mainly in the spike protein, within the RBD and furin cleavage site between S1 and S2, with additional mutations in ORF1ab, ORF8 and the nucleocapsid gene (McCallum et al., 2021; Rambaut et al., 2020). The Beta variant (B.1.351), was first detected in South Africa in October 2020, has RBD mutations

K417N, E484K and N501Y (Tegally et al., 2021). Functional studies showed that mutations N501Y and E484K increased the binding affinity of the RBD to the ACE2 receptor by 7-fold and 1.4-fold respectively, whereas K417N reduced binding by 4-fold (Laffeber et al., 2021). The Gamma variant (P.1), first identified in Brazil in mid-November 2020, has 17 spike mutations, including K417T, E484K and N501Y, which enhanced infectivity (Faria et al., 2021). Compared with non-VOCs, these early variants exhibited increased transmissibility by 29% (Alpha), 25% (Beta), and 38% (Gamma) (Campbell et al., 2021). In May 2021, the Delta variant (B.1.617.2) emerged in India and rapidly became the dominant variant. Delta has a shorter incubation period (4 days compared with 6 days), higher viral loads, and slower viral clearance from the pharynx compared with the ancestral strain (Y. Wang et al., 2021). The reproductive rate of Delta was estimated to be 97% higher than that of non-VOC lineages (Campbell et al., 2021). Later that year, in November 2021, the Omicron variant (B.1.1.529) was reported in South Africa and subsequently spread worldwide. Omicron has over 30 mutations in the spike protein (Ou et al., 2022) and an effective reproductive rate that is 3.19 times higher than that of the Delta variant (Ito et al., 2022).

1.2 Innate immune response to SARS-CoV-2

The innate immune system provides the first line of defence against viral infection. Airway epithelial cells and innate immune cells, including macrophages, dendritic cells (DCs) and natural killer (NK) cells, recognise pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRR), such as retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated gene 5 (MDA5), cyclic GMP–AMP synthase (cGAS) and Toll-like receptors (TLRs) (Kouwaki et al., 2021; Madden & Diamond, 2022; Salvi et al., 2021; Yamada et al., 2021; Zheng et al., 2021). Activation of these pathways induces

pro-inflammatory cytokines and interferons (IFNs), which establish an antiviral state and coordinate immune cell recruitment (Figure 1-2).

SARS-CoV-2 viral proteins, including NSP1, NSP3, NSP6, NSP12, NSP13, NSP14, ORF3, ORF6 and N, suppress host gene translation involved in the innate immune system, such as those encoding IFNs and proinflammatory cytokines (Konno et al., 2020; Kopecky-Bromberg et al., 2007; Lei et al., 2020; Thoms et al., 2020; Vazquez et al., 2021; Xia et al., 2020). These immune evasion mechanisms explain the decrease of IFNα and IFNβ levels observed in severe COVID-19 cases, correlating with poor viral control and heightened proinflammatory responses, such as tumour necrosis factor (TNF) and interleukin (IL)-6, through NF-κB pathway (Hadjadj et al., 2020). Although SARS-CoV-2 suppresses type I IFN induction, the virus remains susceptible to IFNα. In fact, SARS-CoV-2 is more susceptible to type I IFNs, unlike SARS-CoV-1 (Lokugamage et al., 2020).

Host variability in IFN-mediated antiviral responses, driven in part by genetic differences, contributes to heterogeneous COVID-19 severity. Poor clinical outcomes have been linked to defects in type I IFN pathways, including inherited mutations affecting TLR3- and IRF7-dependent IFN production (Zhang et al., 2020), and the presence of neutralising autoantibodies against type I IFNs (Bastard et al., 2020). Patients with autoimmune polyendocrine syndrome type 1 (APS-1), caused by recessive mutations in the autoimmune regulator (AIRE) gene, produce neutralising autoantibodies against type I IFNs which was associated with severe clinical outcomes across age groups (Bastard et al., 2021). These autoantibodies neutralise circulating IFNs, preventing receptor engagement and ISG induction, thereby severely compromising dendritic cell-mediated antiviral responses (van der Wijst et al., 2021). In contrast, certain genetic variants appear protective, for example, a prenylated isoform of 2′-5′-oligoadenylate synthetase 1 (OAS1), an ISG, is associated with

reduced risk of severe COVID-19 (Wickenhagen et al., 2021). OAS1 recognises double-stranded RNA, an intermediate generated during viral replication (Hagemeijer et al., 2012), and activates ribonuclease L (RNase L) to cleave the intracellular viral RNA (Han et al., 2014), thereby inhibiting SARS-CoV-2 replication (Wickenhagen et al., 2021). The protective prenylated OAS1 variant occurs at higher frequency in populations of African ancestry compared with other populations, which may partially contribute to geographic differences in COVID-19 outcomes (Wickenhagen et al., 2021).

Hormonal differences contribute to sex-based variation in innate immunity and divergent COVID-19 outcomes. A meta-analysis reported that male patients had approximately a three-fold higher risk of hospitalisation and death following infection than females (Peckham et al., 2020). Oestrogen and X chromosome dosage increase TLR7-dependent type I IFN production by plasmacytoid dendritic cell (pDC) in females compared with males (Berghöfer et al., 2006; Gilliet et al., 2008; Laffont et al., 2014; Seillet et al., 2012). Conversely, loss-of-function mutations in TLR7 abolish type I and type II IFN responses, a feature reported in young male patients with severe COVID-19 (van der Made et al., 2020). Testosterone also downregulates TLR4 expression on macrophages, leading to reduced TNF production in male mice (Rettew et al., 2008).

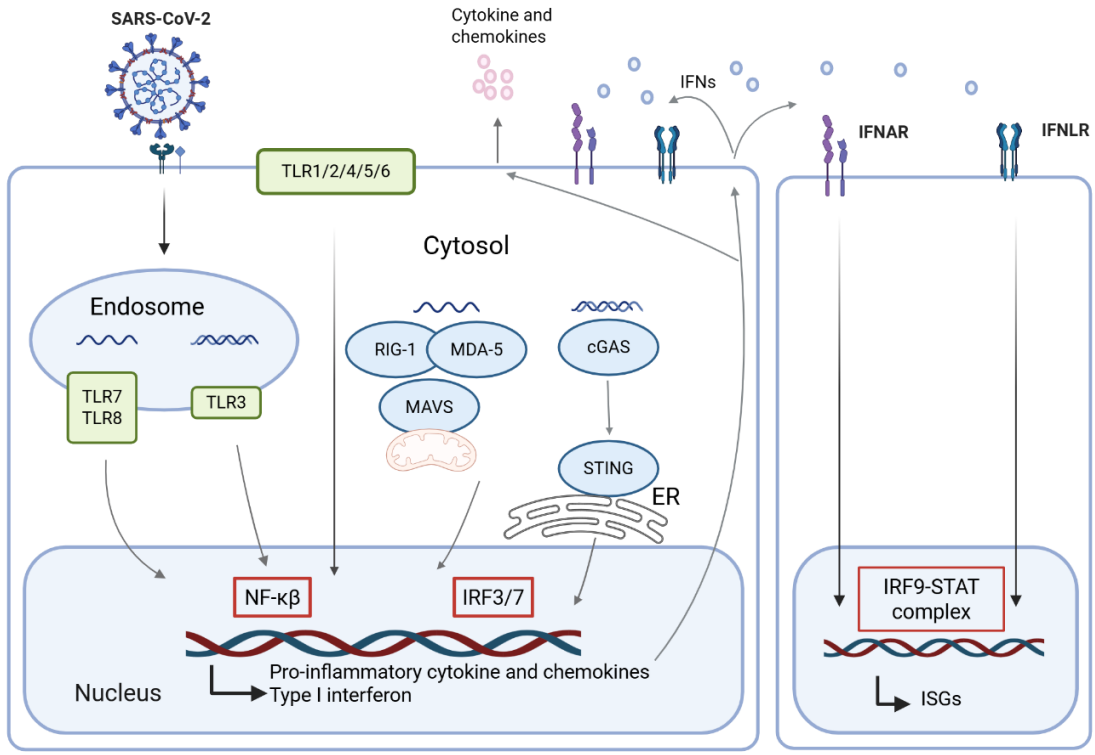

Figure 1-2 Schematic diagram of innate immune sensing

SARS-CoV-2 is detected by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) located at the cell surface and within endosomes, as well as cytosolic RNA sensors of the RIG-I-like receptor (RLR) family (RIG-I, MDA5) and cyclic GMP-AMP synthase (cGAS). Upon detection, transcription factors, such as nuclear factor-κB (NF-κB) and interferon regulatory factor 3 and 7 (IRF3, IRF7), are activated and induce the production of pro-inflammatory cytokines, chemokines, and type I and type III interferons (IFNs). Cytokines and chemokines induce inflammation and recruit immune cells to the sites of infection. IFNs signal through their receptors (IFNAR for type I and IFNLR for type III IFNs) on the same cell or neighbouring cells, activating the Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling cascade. Phosphorylated STAT1 and STAT2 associate with IRF9 to form a complex, translocates to the nucleus and induces transcription of interferon-stimulated genes (ISGs) such as OAS. *Created with Biorender.com.*

1.3 Adaptive immune response to SARS-CoV-2

The second line of defence against pathogens is the adaptive immune response, which is primarily mediated by T and B lymphocytes (Figure 1-3). Unlike innate immunity, adaptive immune responses are antigen-specific and generate long-lived immunological memory.

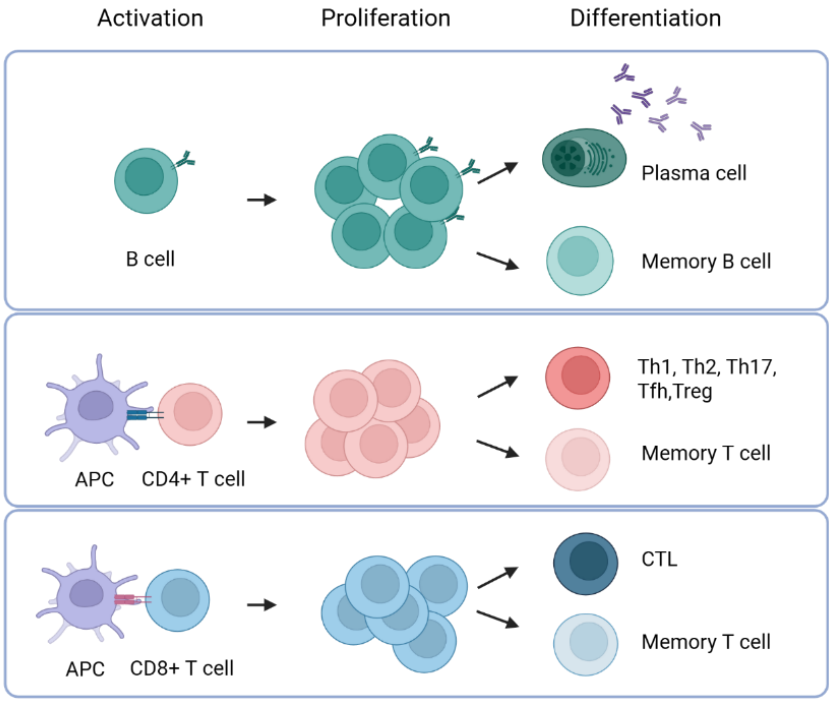

Figure 1-3 Schematic diagram of adaptive immune system

Innate antigen-presenting cells (APCs), such as dendritic cells, capture and process antigen and present peptide-major histocompatibility complex (MHC) complexes to naïve $CD4^+$ and $CD8^+$ T-cells, while B-cells recognise intact antigen. Following activation, B-cells differentiate into antibody-secreting plasma cells and long-lived memory B-cells. Activated T-cells undergo clonal expansion and give rise to effector and memory lineages. $CD4^+$ T-cells differentiate into helper subsets (Th1, Th2, Th17), T follicular helper (Tfh) cells, and regulatory T-cells (Tregs). $CD4^+$ Th1 cells secrete IFN-γ and activate macrophages to control intracellular pathogens, including SARS-CoV-2. Th2 responses secrete IL-4, IL-5, IL-13 targeting eosinophils and are classically induced by helminths. Th17 cells release IL-17 and IL-22, promoting neutrophil responses against extracellular pathogens. T follicular helper (Tfh) cells produce IL-21 to support germinal centre B-cell maturation, class switching and affinity maturation and control extracellular pathogens. Regulatory T-cells (Treg) release suppressive IL-10 and TGFβ to modulate T-cell responses. $CD8^+$ cytotoxic T lymphocytes (CTLs) secrete antiviral cytokines (IFN-γ and TNF) and cytotoxic molecules (granzyme B and perforin) to kill viral infected cells. Memory B and T-cells enable more rapid and robust responses upon antigen re-exposure. *Created with Biorender.com.*

1.3.1 Antibody responses

Naïve B-cells are activated upon binding of antigen to the B-cell receptor (BCR) and receipt of additional signals from helper T-cells. $CD4^+$ T-cells provide costimulatory signals

through CD40-CD40 ligand (CD40L) interactions and the secretion of cytokines. B-cell differentiation occurs in two phases. In the extrafollicular (EF) phase, activated B-cells rapidly differentiate into short-lived antibody-producing plasma cells, predominantly the immunoglobulin (Ig)M, with a subset of cells undergoing class switching to IgG or IgA (Qi et al., 2022). During the germinal centre (GC) phase, B-cells undergo somatic hypermutation and affinity maturation, resulting in the generation of isotype-switched, high affinity plasma cells that are long-lived (Qi et al., 2022).

SARS-CoV-2 nucleocapsid-specific IgM and IgA are detectable in plasma by approximately five days after symptom onset and declined within four weeks (Guo et al., 2020; Wang et al., 2020). IgG antibodies emerge later around day 14 after symptom onset and are maintained for at least six weeks (Guo et al., 2020; Wang et al., 2020). A human challenge study, similarly detected anti-SARS-CoV-2 IgG at day 14 after inoculation (Killingley et al., 2022). The estimated half-life of IgG differs by antigen: ~140 days for spike, ~68 days for nucleocapsid, and ~83 days for RBD (Dan et al., 2021). IgA decays more rapidly, with half-lives of ~14 days against spike and ~27 days against RBD (Dan et al., 2021). Anti-N IgM responses tend to be minimal in mild disease but are more frequently observed in patients with severe diseases (Wang et al., 2020).

The SARS-CoV-2 spike protein is glycosylated (Walls et al., 2020), which partially shields it from antibody recognition. The conformational opening of the spike protein, however, exposes the RBD, making it accessible to antibody binding. The RBD is the most immunogenic region of spike and most of neutralising antibody activity in serum or plasma targets this domain to block viral binding to the ACE2 receptor (Piccoli et al., 2020; Z. Wang et al., 2021). IgG3 and IgM targeting the S1 and RBD are strongly correlated with neutralising antibody (NAb) activity in convalescent plasma (Kober et al., 2022). Despite

having neutralising antibodies, B-cells and circulating follicular helper T-cell (Tfh) against RBD have been reported to be relatively low in patients recovered from COVID-19 (Juno et al., 2020). IgG antibodies directed to RBD display potent avidity at least within one month after symptom onset (Piccoli et al., 2020; Robbiani et al., 2020), even though antibody titres decline over time (Piccoli et al., 2020).

Antibodies to the NTD of spike also exhibit neutralising activity (Chi et al., 2020). However, certain NTD-specific antibodies have been shown to enhance the SARS-CoV-2 infectivity by promoting conformational opening of the RBD, facilitating ACE2 engagement (Y. Liu et al., 2021). This activity is mediated by the $F(ab')_2$ fragments of the antibody (Y. Liu et al., 2021), unlike the case seen in dengue virus infection, which is Fc receptor mediated (Wang et al., 2017). Patients with severe COVID-19 displayed a higher ratio of infection-enhancing to neutralising antibodies compared with non-severe patients (Y. Liu et al., 2021).

Antibody responses contribute to viral clearance during SARS-CoV-2 infection, as increases in plasma antibody levels correlate with declining viral load (Röltgen et al., 2020). Patients with mild disease had detectable viral loads for up to 15 days, while patients with severe disease continued to shed virus for 30-40 days (Wang et al., 2020). Viral shedding is most commonly detected in nasal and throat swabs and sputum, but in severe patients can also be found in faeces, urine and plasma (Wang et al., 2020). In contrast, higher antibody titres have been reported in patients with severe disease compared with those with mild infection. IgG titres against spike and RBD were higher in hospitalised patients than in non-hospitalised ones (Ali et al., 2024; Dan et al., 2021; Piccoli et al., 2020; Robbiani et al., 2020), and memory B-cell frequencies to spike and RBD were also found increased in hospitalised patients (Dan et al., 2021). These elevated humoral responses likely reflect higher antigen burden, whereas early antibody responses to spike and class switching to IgG

were associated with milder disease outcomes (Röltgen et al., 2020; Zohar et al., 2020) (Figure 1-4). In addition, a higher ratio of antibodies against spike or RBD relative to nucleocapsid was more commonly observed in patients who recovered compared with those who did not survive (Atyeo et al., 2020; Röltgen et al., 2020). Although another study did not observe a significant differences in this ratio, it reported that asymptomatic individuals more frequently had antibody to spike than to nucleocapsid, whereas non-survivors were more frequently seropositive for nucleocapsid (Choteau et al., 2022), consistent with nucleocapsid-specific responses reflecting higher viral burden.

Mucosal immunity plays a critical role in defence against SARS-CoV-2, which enters the host primarily through the nasopharynx. The nasopharynx contains nasopharynx-associated lymphoid tissue, a component of the mucosal immune system, where IgA is the predominant antibody isotype at mucosal surfaces. Mucosal and serum IgA originate from distinct compartments. Secretory IgA is produced in mucosal tissues and is polymeric (consist of multiple linked immunoglobulin units), whereas serum IgA is produced in the bone marrow, spleen and lymph nodes and is mostly monomeric (single immunoglobulin units). Polymeric IgA had higher neutralisation activity than monomeric IgA, including more effective in haemagglutination inhibition of influenza virus *in vitro* than monomeric IgA and IgG (Renegar et al., 1998). Secretory IgA in saliva and colostrum was more cross-reactive than serum antibodies (Quan et al., 1997).

SARS-CoV-2 spike IgA in saliva were detected in 70% of convalescent group and can still be detected more than 6 months after infection (Paul et al., 2025). In a human challenge study, SARS-CoV-2 spike IgG, IgA and IgM were detected around day 10 after inoculation (Wagstaffe et al., 2024). Nasal antibodies rose and wanes faster compared to systemic antibodies. IgA is more abundant in saliva than IgG, while IgG was found to be more

abundant in lower respiratory tract in BAL fluid (Sterlin et al., 2021). Neutralisation activity and viral load reduction was correlated strongly with anti-RBD IgA and IgM compared with IgG in saliva or nasal fluid (Sterlin et al., 2021; Wagstaffe et al., 2024).

1.3.2 T-cell responses

T-cells made up ~70% of lymphocytes and are classically divided into helper T-cells ($CD4^+$) and cytotoxic T-cells ($CD8^+$). Activation of naïve T-cells requires T-cell receptor recognition of peptide presented in the context of MHC, and a second signal from innate immune responses, or costimulation. After activation, T-cells proliferate, with $CD8^+$ T-cells proliferating more rapidly than $CD4^+$ T-cells (Foulds et al., 2002). T-cells differentiate into effector and memory T-cells (Figure 1-3).

$CD4^+$ and $CD8^+$ T-cell responses to SARS-CoV-2 can be detected as early as four days post symptom onset (PSO) (Rydyznski Moderbacher et al., 2020). SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T-cells have a half-life of 3-5 months (Dan et al., 2021). During acute infection, CD4+ and CD8+ T-cells were detected in 77% and 50% of COVID-19 patients, respectively (Rydyznski Moderbacher et al., 2020). The presence of $CD4^+$ and $CD8^+$ T-cells targeting the S, M, N, ORF7 and ORF8 was associated with milder disease outcomes (Rydyznski Moderbacher et al., 2020; A. T. Tan et al., 2021).

Following pathogen clearance, effector T-cell numbers contract. $CD4^+$ T-cell contraction is biphasic, with early and late phases, whereas activated $CD8^+$ T-cell contraction is monophasic and occurs at a 10-fold faster rate than that of $CD4^+$ T-cells (De Boer et al., 2003). Memory T-cells persist and can be subdivided according to location and function: central memory T-cells (Tcm) are localised to lymph nodes, while effector memory T (Tem)

cells circulate in the periphery. Effector memory T-cells are reactivated more rapidly upon re-infection. Unlike antibody titres, which wane over time, SARS-CoV-2–specific T-cell responses can persist or even expand after infection, thereby sustaining immune defence as humoral immunity declines (Bilich et al., 2021; Rydyznski Moderbacher et al., 2020). Memory CD8+ T-cells tend to be long-lived, and express CD45RA (Temra), CD127 (IL-7Rα) and T-cell factor 1 (TCF-1) (Adamo et al., 2022), whereas memory $CD4^+$ T-cell numbers decline more gradually over time (Homann et al., 2001).

Dysregulated T-cell responses are a hallmark of severe COVID-19 (Figure 1-4). Severe COVID-19 is characterised by impaired type I IFN signalling, lymphopenia, and defective differentiation of naïve T-cells into effector and memory cells, contributing to poor viral control (Dan et al., 2021; Diao et al., 2020; Hadjadj et al., 2020). In patients with severe disease, the percentage of naive Th cells ($CD3^+CD4^+CD45RA^+$) increases, while memory Th cells ($CD3^+CD4^+CD45RO^+$) decrease (Notarbartolo et al., 2021; Qin et al., 2020). In addition, regulatory T-cells ($CD45RO^+CD3^+CD4^+CD25^+CD127^{low}$) are reduced (Notarbartolo et al., 2021; Qin et al., 2020). These alterations correlate with elevated levels of TNF, IL-6 and IL-10 which promote inflammation, drive T-cell apoptosis and inhibit T-cell proliferation (Diao et al., 2020).

Persistent SARS-CoV-2 viral stimulation contributes to T-cell exhaustion, marked by upregulation of programmed cell death protein 1 (PD-1) and T-cell immunoglobulin and mucin domain containing protein 3 (Tim-3) on $CD8^+$ T-cells, and Tim-3 expression has also been observed on the surface of $CD4^+$ and NK cells (Diao et al., 2020; Hadjadj et al., 2020). Patients with severe disease exhibit reduced functional $CD4^+$ T-cell responses and activated yet exhausted $CD8^+$ T-cells compared with healthy individuals and patients with mild disease (Notarbartolo et al., 2021; Zheng et al., 2020).

Age is one of the strongest risk factors for severe COVID-19: an analysis from New York estimated an infection-fatality risk of ~4.9% for people aged 65–74 years and ~14.2% for those ≥75 years, compared with <1% in younger age groups (Yang et al., 2021). Comorbidities such as diabetes, hypertension, and obesity increased susceptibility to SARS-CoV-2 infection and severe clinical outcomes (Grasselli et al., 2020). Age and chronic conditions promote persistent immune activation which can drive T cells to have senescent phenotypes, characterised by the loss of costimulatory molecule CD28 and the expression of terminally differentiated effector CD57, diminishing *de novo* responses (Coleman et al., 2021). Severe COVID-19 has been associated with increased senescent T cells in elderly patients (Nehme et al., 2020; Zhang et al., 2024).

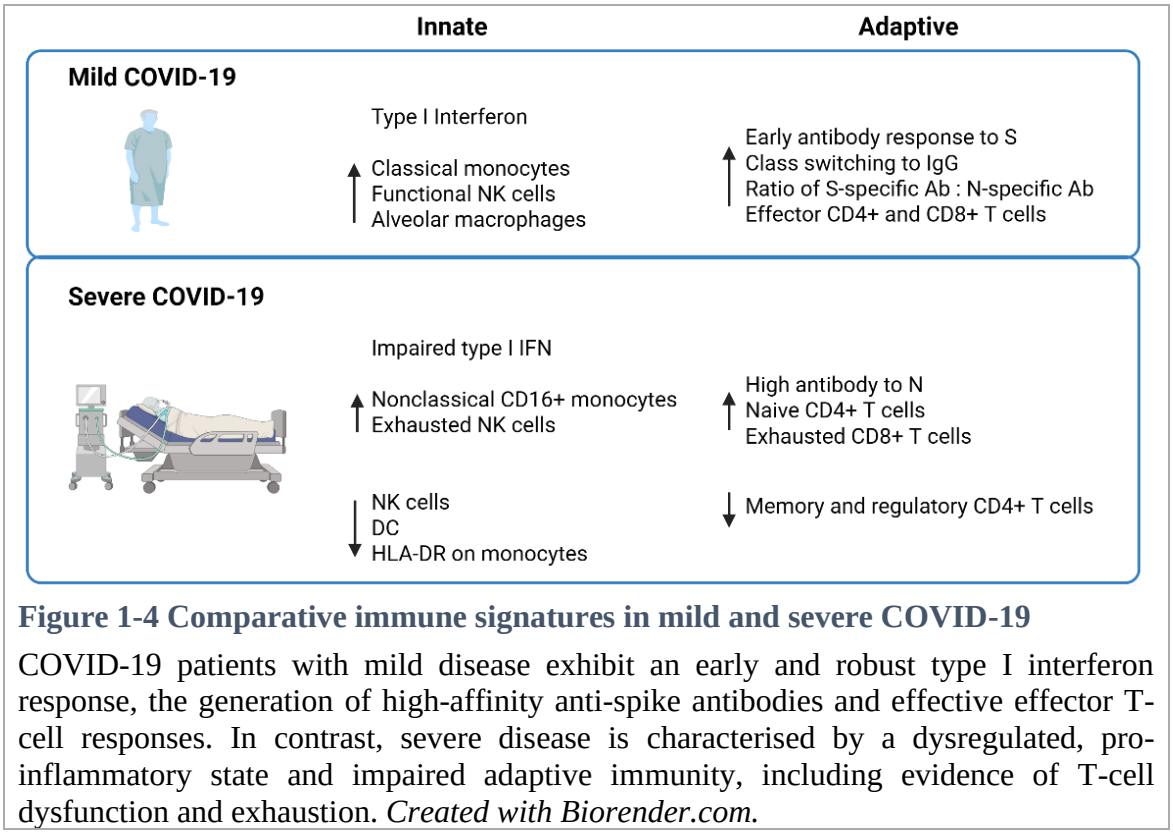

Figure 1-4 Comparative immune signatures in mild and severe COVID-19

COVID-19 patients with mild disease exhibit an early and robust type I interferon response, the generation of high-affinity anti-spike antibodies and effective effector T-cell responses. In contrast, severe disease is characterised by a dysregulated, pro-inflammatory state and impaired adaptive immunity, including evidence of T-cell dysfunction and exhaustion. *Created with Biorender.com.*

1.4 COVID-19 vaccines

Control measures implemented during COVID-19 pandemic, such as wearing masks, lockdowns, contact tracing, screening and physical distancing were in place worldwide to reduce SARS-CoV-2 transmission. Real-world implementation and effects of these interventions however were varied (Alfano et al., 2022; Funke et al., 2023; Masuhara & Hosoya, 2025). Vaccines were developed to more effectively control viral transmission, reduce the morbidity and mortality of COVID-19 and enable the relaxation of lockdown measures (Bauer et al., 2021). The WHO estimated that 13.64 billion COVID-19 vaccine doses had been administered worldwide by 31st December 2023 (World Health Organization, 2024).

By February 2022, four vaccine platform types were approved for emergency use by WHO: mRNA, non-replicating viral vector, inactivated and protein subunit vaccines (VIPER Group COVID19 Vaccine Tracker Team, 2022) (Table 1-1, Table 1-2). Other types of vaccine developed for COVID-19 include DNA vaccines, replicating viral vectors, virus-like particles, replicating viral vectors plus antigen presenting cells, non-replicating viral vectors plus antigen presenting cells, live attenuated viruses and bacterial antigen-spore expression vectors (World Health Organization, 2023).

Table 1-1 Characteristics of COVID-19 vaccine platforms

Feature	mRNA	Viral vector	Inactivated	Protein subunit
Dose per vial	30 μg in 0.3 mL (BNT162b2); 100 μg in 0.5 mL (mRNA-1273)	$5x10^{10}$ viral particles in 0.5 mL (AZD1222, Ad26.COV2 S); $5x10^{10}$ viral particles in 0.5 mL (Ad5-nCoV)	3 μg in 0.5 mL (CoronaVac), 4 μg in 0.5 mL (BBIBP-CorV), 6 μg in 0.5 mL (BBV152)	5 μg protein and 50 μg Matrix-M adjuvant (NVX-CoV2373); 25 μg protein (0.25 mL) and AS03 adjuvant (0.25 mL) (GBP510/AS03)

Storage	-70°C (BNT162b2); -20°C (mRNA-1273); 2°C to 8°C for up to 30 days	2°C to 8°C	2°C to 8°C	2°C to 8°C
Ease of manufacture	Rapid manufacturing, scalable, cell-free production	Scalable; uses cell culture for vector growth.	Established technology; requires virus propagation and inactivation	Complex recombinant protein production; requires adjuvants
Cost	High	Low-Moderate	Low	Moderate

1.5 Immune responses to different COVID-19 vaccine platforms after the primary series

Immunity to SARS-CoV-2 is associated with the induction of systemic neutralising antibody and T-cell responses against the spike protein (Brady et al., 2025; Goldblatt et al., 2022; Khoury et al., 2021). Neutralising antibodies bind to SARS-CoV-2 spike, inhibiting viral entry into host cells, by blocking the interaction between RBD and ACE2 receptor on the host cells (Piccoli et al., 2020; Z. Wang et al., 2021). By preventing viral binding or fusion, neutralising antibodies provides early protection against infection. Neutralising activity using live virus is labour intensive and high-level biosafety containment. Alternative approaches that can be used includes pseudovirus neutralisation assay and surrogate ACE2 inhibition binding assay to estimate neutralisation capacity. The level of binding antibody in the blood do not directly measure functional capacity, because not all antibody block with viral entry. However, the level of IgG and IgA to spike, RBD or NTD have been shown to be correlated with neutralising activity against SARS-CoV-2 infection (Chi et al., 2020; Hertz et al., 2023; Piccoli et al., 2020; Sterlin et al., 2021; Z. Wang et al., 2021).

Protection against severe disease outcome following viral infection is mediated by T-cells. Effective vaccine-induced cellular immunity depends on of the presence of naïve T-cells to recognise antigenic epitopes in the vaccine are needed to generate memory T-cell following vaccination (Wang et al., 2023). Naïve T cells become activated, proliferate and differentiate into memory cells. Memory T-cells generated by vaccination can then be rapidly activated following viral exposure, limiting viral replication and spread. The development of adaptive responses also requires the initial activation of innate immune responses. Different components of COVID-19 vaccines induce innate and adaptive immune responses with varied efficacy (Table 1-2, Table 1-3). This section reviews immune responses following completion of the primary vaccination series.

Table 1-2 Immune responses and efficacies of COVID-19 vaccine platforms approved for emergency use by WHO

Type of vaccines	Vaccines	Antigen	Innate sensor	Adaptive immune responses (Phase I/II)	Efficacy after completion of the primary vaccination series (Phase III)
a. mRNA 	• Moderna-Spikevax (mRNA-1273) • Pfizer-BioNTech-Comirnaty (BNT 162b2)	Spike	mRNA: TLR7 and MDA5 Lipid nanoparticle: NLRP3 inflammasome	NAb, Th1 $CD4^+$ T-cell, $CD8^+$ T-cell	• 94.1% (Baden et al., 2021) • 95% (Polack et al., 2020)
b. Viral vector 	• Oxford/AstraZeneca/Vaxzervria (ChAdOx1 nCoV-19 (AZD1222)) • Serum Institute of India-Covishield (Oxford/AstraZeneca formulation) • CanSino-Convidecia (Ad5-nCoV) • Janssen (Johnson & Johnson)-Jcovden (Ad26.COV2.S)	Spike	adenoviral vector and double-stranded DNA: TLR9, cGAS/STING	NAb, Th1 $CD4^+$ T-cell, $CD8^+$ T-cell	• 64% (Falsey et al., 2021) • 57.5% (Halperin et al., 2022) • 66.9% (J. Sadoff et al., 2021)
c. Whole inactivated 	• Bharat Biotech-Covaxin (BBV152) • Sinopharm (Beijing)-Covilo (BBIBP-CorV) • Sinovac-CoronaVac	Whole virus	SARS-CoV-2: TLR7/8 Alum: NLRP3 inflammasome	NAb, Th1 and Th2 $CD4^+$ T-cell	• 77.8% (Ella et al., 2021) • 79% (Al Kaabi et al., 2021) • 65.3% - Indonesia (Fadlyana et al., 2021), 83.5% - Turkey (Tanriover et al., 2021)
d. Protein subunit 	• Novavax-Nuvaxovid (NVX-CoV2373) • Serum Institute of India-COVOVAX (Novavax formulation) • SK Bioscience-SKYCovione (GBP510/AS03)	Spike RBD	rSARS-CoV-2 Matrix-M: NLRP3 inflammasome, Matrix induced damage-associated molecular patterns and cytokines (Syk- and cathepsin B-dependent cell activation)	NAb, Th1 $CD4^+$ T-cell	• 96.4% non-Alpha variant (Heath et al., 2021) • NAb superiority to ChAdOx1-S (Song et al., 2023)

1.5.1 mRNA vaccines

mRNA vaccines consist of lipid nanoparticle (LNP)-encapsulated viral mRNA incorporating modified nucleotides. Once delivered into host cells, the mRNA is released into the cytosol, where it is translated by the host ribosomal machinery into the SARS-CoV-2 spike protein. The incorporation of modified nucleotides, methylated pseudouridine, reduces endolysosomal nuclease-mediated RNA degradation and limits recognition by TLR7 and TLR8 preserving mRNA stability (Bérouti et al., 2025). The LNP component of mRNA vaccines contributes to innate immune activation, through inflammasome activation and cytokine induction (Kedmi et al., 2010; H. Zhang et al., 2021).

The newly synthesised spike protein is subsequently processed through intracellular antigen-processing pathways and presented on MHC molecules to T-cells. The mRNA-1273 vaccine induces $CD4^+$ T-cell responses polarised towards a Th1 responses, with TNF production predominating, followed by IL-2 and IFN-γ secretion (Jackson et al., 2020). $CD4^+$ Th2 cytokines, IL-4 and IL-13, and $CD8^+$ T-cell were also detected but at lower levels (Jackson et al., 2020). Similarly, the BNT162b2 vaccine induces IFN-γ-secreting $CD4^+$ and $CD8^+$ T-cells specific to S1 (N-terminal) and S2 (C-terminal) (Sahin et al., 2021). IFN-γ producing T-cells contract after 29 days, but remain detectable at day 85, indicating sustained cellular immunity (Sahin et al., 2021). Longer dosing intervals between mRNA vaccine doses have been shown to reduce the inflammatory environment and potentially promoted the generation of T-cells with greater proliferative and recall potential, compared with short-interval regimens, suggesting that extended intervals can optimise cellular immunity (Murray et al., 2025; Payne et al., 2021).

Vaccines confer protection by generating antibody-secreting plasma cells and memory B-cells, processes that depend on Tfh cells within secondary lymphoid organ (Crotty, 2011).

Analysis of draining axillary lymph nodes from recipients of the BNT162b2 showed the induction of spike-specific Tfh cells (Lederer et al., 2022; Mudd et al., 2022; Turner et al., 2021). In mice, Tfh activation can be triggered by IL-6, which is induced in part by the LNP component of mRNA vaccines (Zhang et al., 2022). Tfh responses peaked after one week following the second dose and persisted for 6 months (Mudd et al., 2022; Turner et al., 2021). Monoclonal antibodies from germinal centre B-cells were mostly directed against the RBD protein, although some exhibited cross-reactivity with seasonal coronaviruses HCoV-OC43 and -HKU1 (Turner et al., 2021).

Neutralising antibody titres after vaccination have been correlated with protection from SARS-CoV-2 infection (Earle et al., 2021; Khoury et al., 2021). Both mRNA vaccines, mRNA-1273 and BNT162b2, induce neutralising antibody responses (Z Wang et al., 2021). mRNA-1273 induces higher and more durable humoral immunity than BNT162b, likely reflecting the higher antigen dose (100 ug/0.5 mL vs 30 ug/0.3 mL) and longer dosing interval (28 days vs 21 days). Besides neutralisation, antibodies have effector functions mediated by their Fc-region that engages innate immune cells, such as antibody-dependent neutrophil phagocytosis (ADNP), antibody-dependent monocyte phagocytosis (ADMP), antibody-dependent complement deposition (ADCD), and antibody-dependent cellular cytotoxicity (ADCC). mRNA-1273 vaccine elicits stronger antibody-dependent neutrophil phagocytosis and natural killer cell activation than BNT162b2, while both vaccines induce comparable antibody-dependent complement deposition and antibody-dependent monocyte phagocytosis (Kaplonek et al., 2022).

Approximately 30% of vaccine recipients developed spike-specific secretory IgA, and 58% showed RBD-specific IgA responses in saliva after two doses of mRNA vaccines, BNT162b2 and mRNA-1273 (Sheikh-Mohamed et al., 2022). IgA induction in saliva and

bronchoalveolar lavage fluid (BAL) was modest in SARS-CoV-2 naïve individuals, whereas mucosal antibodies were boosted in previously infected individuals following vaccination (Liew et al., 2023; Sano et al., 2022; Tang et al., 2022), indicating the influence of baseline immunity on vaccine responsiveness. The mechanism underlying mucosal immunity after intramuscular mRNA vaccination is still unclear. It is thought that APCs in peripheral lymph nodes, or the antigen-specific B-cells, or the antigen from the vaccine migrate to mucosal sites and induce mucosal immune responses (Sano et al., 2022). Another speculation is that the responses came from cross-reactive memory cells elicited by seasonal coronaviruses (Sano et al., 2022).

Repeated vaccination with SARS-CoV-2 mRNA vaccines induces an increase in spike-specific IgG4 antibodies (Buhre et al., 2022; Irrgang et al., 2023; Kalkeri et al., 2024), which was not observed with the viral vector vaccine AZD1222 (Buhre et al., 2022) or the protein sub-unit vaccine, NVX-CoV2373 (Irrgang et al., 2023). The mechanisms driving IgG4 induction by mRNA vaccinations and its implications are still unclear. IgG4 is the least abundant IgG subclass (<5% of total IgG) and is associated with reducing chronic inflammation and immune tolerance (Rispens & Huijbers, 2023). Compared with other IgG subclasses, IgG4 shows limited Fc-mediated effector functions (Tam et al., 2025). Although spike-specific IgG4 can inhibit Fc-mediated antibody functions through epitope competition in monoclonal systems, IgG4 targets only a subset of spike epitopes and does not prevent functional IgG1 and IgG3 antibodies from mediating effector responses to different epitopes (Tam et al., 2025).

1.5.2 Viral vector vaccines

Viral vector vaccines are immunostimulatory because the adenoviral vector and the double-stranded DNA activate innate immune pathways, including TLR9 inducing type I IFN

production (Appledorn et al., 2008; Teijaro & Farber, 2021). In murine models, recombinant adenovirus type 5 (Ad5) vectors triggered the cGAS/stimulator of interferon genes (STING) pathway, promoting IFN-β secretion and inflammatory chemokine induction (Anghelina et al., 2016). pDC-derived IFN-α and IL-6 and activated mucosal-associated invariant T (MAIT) cells, contributed to immunogenicity following adenoviral vector vaccination (Provine et al., 2021; Pudjohartono et al., 2025). SARS-CoV-2 spike gene delivered by viral vectors is transcribed and translated, then processed and presented via MHC, activating T-cells. Viral vector vaccines face challenges to be deployed for repeated administration as the host can generate immune responses to the viral vector used. ChAdOx1 nCoV-19 (AZD1222) uses replicating-deficient adenoviral vectors from simian to minimise human pre-existing immunity, whereas Ad5-nCoV vaccine (CanSino) and Ad26.COV2.S (Janssen) uses human adenoviruses. In the Phase 1 study of Ad5-nCoV, about 50% of participants had pre-existing NAb to Ad5, reducing seroconversion rates and IFN-γ T-cell responses post vaccination with CanSino Ad5-nCoV (F. C. Zhu et al., 2020).

Single dose or two dose viral vector vaccines induced robust spike-specific IgG and NAb following the primary vaccination (Barrett et al., 2021; Jerald Sadoff et al., 2021). Neutralising antibody activity following ChAdOx1 nCoV-19 vaccination correlated strongly with anti-spike antibody levels (Folegatti et al., 2020). After two doses, ChAdOx1 induced lower anti-spike IgG titres than the mRNA vaccine BNT162b2 (Moore et al., 2023; Wei et al., 2022), but higher titres than the inactivated vaccine CoronaVac (Harris et al., 2024). Protection against SARS-CoV-2 infection following ChAdOx1 vaccination persisted for approximately 2-3 months, which is roughly half the duration observed after two doses of mRNA vaccines (Wei et al., 2022). Viral vector vaccines also generated Fcγ-receptor-binding (type I, IIa, and IIIa/b) antibodies (Harris et al., 2024). Antibody-dependent phagocytosis activity, mediated by monocytes and neutrophils, was higher in the vaccinated

group than in convalescent patients. Antibody-dependent NK cell activation and complement activity were also increased after vaccination, reaching levels similar to those in convalescent plasma (Folegatti et al., 2020). Mucosal IgG and IgA were detected in bronchoalveolar lavage fluid following two or three doses of ChAdOx1 nCoV-19 vaccines, however no memory B cells were detected (Mitsi et al., 2023). Intramuscular vaccination however can enhance the mucosal memory cells in the airway in those previously infected (Mitsi et al., 2023).

Comparison of memory B-cells after vaccination showed that high frequencies of CXCR3+ memory B-cells against spike were detected following a single dose of the viral vector vaccine Ad26.COV2.S at 6 months but not following mRNA vaccines (Zhang et al., 2022). $CXCR3^{+}$ facilitates B-cell migration to infected areas where they differentiate and secrete antibody. Compared with viral vector vaccines, mRNA vaccines induce higher frequencies of activated memory B-cells ($CD21^{-}CD27^{+}$) and proliferating B-cells ($CD71^{+}$), a phenotype consistent with enhanced germinal centre–associated B-cell responses (Zhang et al., 2022).

Viral vector vaccines also induce cellular immunity. IFN-γ-secreting T-cells were detected by day 14 after the first dose (Folegatti et al., 2020; Halperin et al., 2022; Jerald Sadoff et al., 2021; F. C. Zhu et al., 2020). T-cell responses declined 1 month after single dose vaccine (F. C. Zhu et al., 2020), whereas the second dose of ChAdOx1 nCoV-19 vaccine-maintained T-cell responses (Folegatti et al., 2020; Halperin et al., 2022; F. C. Zhu et al., 2020). $CD4^{+}$ Th1-skewed responses were detected with spike-protein specific TNF secreting cells being the most frequent, followed by IL2 and IFN-γ, at day 28 after two doses (Swanson et al., 2021; F. C. Zhu et al., 2020). The polyfunctionality of $CD4^{+}$ T-cells increased after the second dose of ChAdOx1 nCoV-19, even though the magnitude did not increase (Swanson et al., 2021). Th2 responses remained minimal (Swanson et al., 2021). $CD8^{+}$ T-cells

responses were less frequent than $CD4^+$ responses, with IFN-γ the dominant cytokine, followed by TNF and IL2 (Swanson et al., 2021). Only 30% participants aged ≥65 years had S-specific $CD8^+$ IFN-γ^+ and IL-2^+ after single dose Ad26.COV2 S (Jerald Sadoff et al., 2021). $CD8^+$ T-cells were markedly boosted in those aged ≥65 years following ChAdOx1 nCoV-19 vaccine, emphasising the benefit of additional dosing to enhance cellular immunity in this age group (Swanson et al., 2021).

1.5.3 Inactivated vaccines

Inactivated vaccines require the addition of adjuvants to enhance their immunostimulatory properties. CoronaVac, for example, uses aluminium hydroxide (AH) as its adjuvant. AH activates the NLRP3 inflammasome, which is abundantly expressed in macrophages, leading to the release of pro-inflammatory cytokines, IL-1β and IL-18 (Eisenbarth et al., 2008). Both IL-1β and IL-18 belong to the IL-1 family that signal through IL-1 receptors (IL1R) on lymphocytes. $CD4^+$ T-cells require IL-1 signalling to secrete effector cytokines, despite being capable of differentiating into Th1, Th2 and Th17 cells upon TCR ligation (Jain et al., 2018). AH also slowed protein degradation within dendritic cells and enhanced antigen presentation via MHC class II (Ghimire et al., 2012), promoting $CD4^+$ T-cell differentiation. *In vivo* studies showed that aluminium adjuvants skewed $CD4^+$ T-cells towards a Th2 response, producing IL-4 (Serre et al., 2010).

IL-1β also promotes $CD8^+$ T-cell differentiation towards granzyme B^+ and IFN-γ^+ effector phenotypes (Ben-Sasson et al., 2013), but aluminium adjuvant alone elicits IFN-γ^+ $CD8^+$ T-cells with limited cytotoxic capacity (Serre et al., 2010). The addition of a TLR4 agonist such as monophosphoryl lipid A (MPL) enhanced antigen cross-presentation and promoted the generation of cytotoxic $CD8^+$ T-cells (MacLeod et al., 2011), a component not included in currently deployed inactivated vaccines. The combination of AH and MPL promotes

cytotoxic CD8+ T-cells differentiation by downregulating PD-1, in the presence of IL-6, leading to the increase in granzyme B expression (MacLeod et al., 2011). In addition, effector memory B-cells express IL-1R and IL-18R (Jain et al., 2018), supporting the adjuvant role in promoting the secretion of antigen specific antibody (Eisenbarth et al., 2008).

In a Phase I/II study of BBIBP-CorV and CoronaVac, inactivated vaccines induced NAb against SARS-CoV-2 (Xia et al., 2021; Y. Zhang et al., 2021). The durability, however, was short-lived as NAb were no longer detected after 90 days (J. Liu et al., 2021). Individuals who developed anti-spike IgA seropositivity after CoronaVac vaccination were more likely to retain detectable neutralising antibody at day 160 (Xu et al., 2021). Compared with the adenoviral vector vaccine ChAdOx1 nCoV-19, CoronaVac recipients exhibited higher spike-specific IgM (Harris et al., 2024). IgM, a pentameric immunoglobulin, efficiently fixes complement via C1q binding (Cooper et al., 1983), a property confirmed for infection-derived immune responses (Harris et al., 2024). In contrast, in vaccinated individuals, complement fixation correlated more strongly with IgG than IgM (Harris et al., 2024). IgG requires interactions with other IgG molecules to form hexamer to activate C1q (Diebolder et al., 2014). The IgG subclasses that contribute to C1q-fixation for activating classical pathway depend on antigen concentration (Garred et al., 1989; Michaelsen et al., 1991). IgG1 activated C4 and C3 more effectively at high antigen concentrations, while IgG3 is more effective at lower antigen concentrations (Garred et al., 1989). Inactivated vaccines induced $CXCR5^{+}$ B-cells expression indicating resting memory B-cells (Nuñez et al., 2023), unlike mRNA and viral vector vaccines which generated activated memory B-cells (Zhang et al., 2022), showing the vaccine platform differences in B-cell recall potential.

Inactivated vaccines can induce immune responses to both structural and non-structural SARS-CoV-2 proteins, providing broader antigenic coverage than spike-only vaccines. Two doses of CoronaVac induced higher $CD4^{+}IFN\text{-}\gamma^{+}$ responses to combined spike, nucleocapsid, envelope and membrane antigens than BNT162B2 one month after completion of the primary vaccination series (Lim et al., 2022; Mok et al., 2022). Activated and memory $CD4^{+}$ T-cells against spike were detected 14-30 days after the second CoronaVac dose and persisted for 75-165 days (Costa et al., 2022). T-cells to non-spike antigens were detected at 75-165 days post second dose (Costa et al., 2022). Consequently, $IFN\text{-}\gamma^{+}CD4^{+}$ and $CD8^{+}$ T-cells to nucleocapsid were not significantly different from those in non-vaccinated individuals when measured one-month post-vaccination (Q. Peng et al., 2022). $CD4^{+}$ $CD40L^{+}$ Tfh ($CD45RA^{-}CXCR5^{+}$) cells, which modulate B-cell maturation (Crotty, 2014), were predominantly elicited to spike antigen and peaked 14 days after vaccination (Costa et al., 2022). $CD4^{+}$ Tcm ($CCR7^{+}CD45RA^{-}$) and Tem ($CCR7^{-}CD45RA^{-}$) cells to RBD or spike and nucleocapsid, were detected after vaccination, but Temra ($CCR7^{-}CD45RA^{+}$) was not significantly expanded (Castro et al., 2022; Costa et al., 2022). These memory cells remained detected after 12 months although at low level (Zhao et al., 2022). CoronaVac induced higher spike-specific $CD4^{+}$ Tem than Tcm compared with BNT126B2, a phenotype that may delay recall responses upon reinfection (Mok et al., 2022). Inactivated vaccines predominantly induce $CD4^{+}$ T-cells, while mRNA and viral vector vaccines can generate both $CD4^{+}$ and $CD8^{+}$ T-cells (Lim et al., 2022; Swanson et al., 2021). Single cell transcriptomics analysis of T-cell subsets after two doses of inactivated vaccine showed reduced frequencies of proliferating $CD8^{+}$ T-cells and regulatory $CD4^{+}$ T-cells (J. Liu et al., 2021).

$CD4^{+}$ T-cells produced Th1 cytokines after the first inactivated vaccine, with a shift towards Th2 cytokines after the second dose (Jiang et al., 2022). CoronaVac skewed toward a Th2

responses, while mRNA and viral vector vaccines had Th1 polarized responses (Padilla-Bórquez et al., 2024). Th2-type $CD4^+$ T-cells ($CD3^+CD4^+IL\text{-}4^+$) generated following primary vaccination with inactivated vaccine persisted after a booster with BNT162B2 (Phoksawat et al., 2022).

1.5.4 Protein subunit vaccines

Purified recombinant proteins alone do not have the microbial patterns to induce robust innate immune responses. In protein subunit vaccines, adjuvants are added to promote recruitment of APCs to the injection site and transport the antigen to draining lymph nodes (Stertman et al., 2023). Adjuvant and antigen localised to lysosomes (Stertman et al., 2023). Saponin induced the production of IL-1β and IL-18 via NLRP3 inflammasome activation (Stertman et al., 2023). Other cytokines produced through NF-κB signalling pathways are TNF, IL-6, IL-8. Antigens released from lysosomes can be processed or remain intact and be presented to $CD4^+$ and $CD8^+$ T-cells (Stertman et al., 2023).

The Novavax vaccine, NVX-CoV2373, is a recombinant nanoparticle containing full-length wild-type SARS-CoV-2 spike protein (Keech et al., 2020). The saponin-based adjuvant, Matrix-M1, induced NAb and $CD4^+IFN\text{-}\gamma^+$, TNF^+ or $IL\text{-}2^+$, with limited IL-5 and IL-13 secretion, indicating Th1 skewed responses (Keech et al., 2020). Without adjuvant, $CD4^+$ T-cells responses were very limited (Keech et al., 2020). SK Bioscience GBP510 uses AS03, which contains α-tocopherol and squalene. In a pre-clinical study in rhesus macaques, the RBP nanoprotein with AS03 generated balanced Th1 and Th2 CD4 T-cells (Arunachalam et al., 2021). In a clinical study, GBP510 generated higher NAb levels than ChAdOx1-S (Song et al., 2023).

Table 1-3 Relative induction of immune responses by COVID-19 vaccine platforms

Immune responses	**mRNA vaccines**	**Viral vector vaccines**	**Inactivated vaccines**	**Protein subunit vaccines**
Spike-specific Abs	+++	++	+	++
NAb activity durability	+++	++	+	++
Non-spike-specific Abs	-	-	+++	-
Spike-specific $CD4^+$ T-cell responses	+++	+++	++	++
Spike-specific $CD8^+$ T-cell responses	++	+++	+	+

Legend: + (low induction), ++ (moderate induction), +++ (strong induction), - (not observed)

1.6 Immune responses against variants after vaccination

Beside platform-dependent differences in the magnitude and quality of vaccine-induced immunity, the emergence of SARS-CoV-2 variants highlighted an additional challenge related to antigenic specificity (Hacisuleyman et al., 2021; Kustin et al., 2021; Z Wang et al., 2021). COVID-19 vaccines were developed against the ancestral SARS-CoV-2 strain, and the emergence of viral variants with epitope mutations led to reduced vaccine-induced neutralisation (Z Wang et al., 2021). In combination with waning immunity, this raised concerns regarding breakthrough infections and continued viral transmission.

Nab generated by COVID-19 vaccines were reduced by 2-fold against the B.1.1.7 (Alpha) variant and 4.5-6.7-fold against the P.1 (Gamma) variant (Karim & Oliveira, 2021). Individuals with lower NAb a week before infection exhibited higher viral load during B..1.1.7 breakthrough infections (Bergwerk et al., 2021), indicating the importance of maintaining antibody levels following vaccination, despite reduced neutralisation activity against variants. Fc-mediated antibody effector functions are less dependent on epitope specificity, thus vaccine-induced antibodies, such as those generated by mRNA vaccines,

can still play role in clearing Alpha, Beta, and Gamma variants, albeit non-neutralising (Kaplonek et al., 2022).

The increased transmissibility of the Omicron variant also showed the need of population-level vaccine coverage for limiting viral transmission. In Denmark, despite 76% of the population being fully vaccinated with two doses and another 7.1% having received three doses, the Omicron variant reproduction rate was three times higher than Delta and accounting for more than 95% of SARS-CoV-2 infection in December 2021 (Ito et al., 2022). However, the transmissibility of Omicron over Delta variant was even greater in South Africa (4.2-fold), where vaccination coverage was around 30% at that time (Nishiura et al., 2022). Despite increased transmissibility, Omicron infection was associated with reduced severity and lower rates of hospitalisation compared with Delta (Sheikh et al., 2022; Wolter et al., 2022).

In response to emerging variants and waning immunity, booster doses were incorporated into COVID-19 vaccination recommendations to sustain high levels of immunity. Administration of a booster dose increased protection against SARS-CoV-2 infection in 2022 compared with two doses of vaccine (Sheikh et al., 2022). In addition, updated mRNA vaccines incorporating spike sequences from circulating variants were deployed from August 2022. These bivalent vaccines contained two spike components, one derived from the ancestral strain and another from Omicron subvariants BA.4 and BA.5. The bivalent vaccines demonstrated 59-72% effectiveness against hospitalisation and 62-68% effectiveness against death in adults aged ≥18 years and ≥65 years, respectively, during periods of Omicron circulation (Arbel et al., 2023; D. Y. Lin et al., 2023). Bivalent vaccines conferred protection not only against BA.4 and BA.5, but also against other Omicron subvariants, including BQ.1-BQ.1.1 and XBB.1.5 (D. Lin et al., 2023).

1.7 Immune responsiveness to COVID-19 vaccination

Although vaccine platform shaped the quality and breadth of immune responses, or immunogenicity, host immune history and baseline immune state or immunocompetence, influenced individual vaccine responsiveness. Results from a human SARS-CoV-2 challenge study demonstrated that heterogeneity in host immune responses was associated with divergent infection outcomes, as reflected by how long the infection was sustained (Lindeboom et al., 2024). Individuals with transient infection showed early innate and adaptive immune responses, including T-cell which were detected in the nasopharynx as early as day 1 following inoculation, whereas those with sustained infection exhibited delayed T-cell responses, emerging at day 5 and peaking at day 10 (Lindeboom et al., 2024). Consistent with this, observational studies reported that rapid T-cell responses following natural infection were observed in those with mild disease and viral clearance (Rydyznski Moderbacher et al., 2020; A. T. Tan et al., 2021). The clinical relevance of T-cell heterogeneity is further shown in a placebo-controlled trial, T-cell responses following Ad26.COV2.S COVID-19 vaccination were correlated with a reduce risk of severe COVID-19 (Hertoghs et al., 2025), indicating that variability in vaccine-induced cellular immunity influences disease progression.

Potential factors influencing heterogeneous vaccine responses in healthy individuals includes age, sex, genetics and prior antigenic exposure (Falahi & Kenarkoohi, 2022; Zimmermann & Curtis, 2019). With increasing age, the immune system decline in function accompanies by low grade inflammation that impairs vaccine responsiveness, especially in elderly population. Oestrogen and X chromosome dosage increase TLR7-dependent type I IFN responses by plasmacytoid dendritic cell (pDC) in females compared to males (Berghöfer et al., 2006; Gilliet et al., 2008; Laffont et al., 2014; Seillet et al., 2012),

influencing how host immune response recognise the vaccine antigen. Polyphormisms in human leukocyte antigen (HLA) genes, which are responsible for antigen presentation, influence the T-cell binding to antigens in vaccine. HLA alleles have been associated with vaccine-induced immune responses following COVID-19 vaccination and risk of breakthrough infection (Astbury et al., 2022; Mentzer et al., 2023; Xie et al., 2024).

Genetic and acquired immune disorders can impair adaptive immune responses, affecting vaccine immunogenicity. Studies in immunocompromised patients, including those with inborn errors of immunity or those with acquired immune disorders showed heterogeneity in vaccine-induced immune responses (Gao et al., 2022; Goodyear et al., 2024; Shields et al., 2022). Patients with X-linked agammaglobulinemia (XLA) elicited T-cell responses following mRNA vaccination, while those with solid-organ transplant and chronic leukaemia patients had impaired T-cell responses (Gao et al., 2022). Characteristics of T-cell in haematological malignancies includes impaired cytokine production, poor proliferation activity, exhaustion markers, increased regulatory T cells (Tao et al., 2024; Yoo et al., 2025). Additional vaccine doses have been shown to induce or enhance vaccine-induced immunity in this population (Fendler et al., 2022; Goodyear et al., 2024; Schrezenmeier et al., 2021; Shields et al., 2022). The timing of the additional vaccine dose is also critical. For example, delaying the additional vaccine dose after treatment of transplantation in hematologic cancer patients improved humoral responses (Haggenburg et al., 2022). Hence, vaccine strategies in immunocompromised individuals should take into account the underlying immune condition and treatment to induce effective immune responses.

In this section, I review evidence that prior exposure to seasonal human coronaviruses and features of immune ageing, immunosenescence, associated with age and persistent infection, modulate immune responses to SARS-CoV-2 vaccination.

1.7.1 Pre-existing cross-reactive immune responses in unexposed populations

About 20-50% of individuals with no known exposure to SARS-CoV-2 had T-cells that are cross-reactive with SARS-CoV-2 antigens (Anderson et al., 2021; Grifoni et al., 2020; Le Bert et al., 2020). These pre-existing SARS-CoV-2-reactive T-cells are thought to arise from prior exposure to endemic seasonal human coronaviruses, although contributions from other viral infections or vaccinations cannot be excluded.

Seven coronaviruses are known to infect humans (Figure 1-5). Alphacoronaviruses (Human coronavirus (HCoV)-229E and -NL63) and betacoronaviruses (HCoV-OC43 and -HKU1) are endemic seasonal coronaviruses that cause mild respiratory illnesses. In contrast, SARS-CoV, Middle East respiratory syndrome (MERS) and SARS-CoV-2 have caused epidemic outbreaks associated with more severe disease, including pneumonia. Reported overall sequence homology between SARS-CoV-2 and seasonal coronaviruses ranges from 65% for alphacoronaviruses to 69% for betacoronaviruses (Kaur et al., 2021). SARS-CoV-2 is more closely related to other highly pathogenic coronaviruses, MERS and SARS-CoV, sharing 69.58% and 82.45% homology, respectively (Kaur et al., 2021) (Figure 1-5). Certain viral regions are relatively conserved across coronaviruses, including the S2 subunit of spike, as well as the membrane, nucleocapsid and ORF1 proteins (Kaur et al., 2021; Murray et al., 2023). SARS-CoV-2 spike displays lower homology with the seasonal HCoVs (OC43 29.54%, HKU1 28.42%, NL63 25.45%, 229E 25.29%) and MERS-CoV (30.81%), but high similarity with SARS-CoV (76.04%) (Geanes et al., 2022).

Longitudinal analyses of HCoVs indicate that immunity wanes within 12 months, allowing reinfection with the same strain (Grifoni et al., 2020; Le Bert et al., 2020). Evidence for partial cross-protection among seasonal HCoVs has been observed in studies of newborns (Dijkman et al., 2012). Similarly, cross-reactive immune responses have been reported among SARS-CoV, SARS-CoV-2, and MERS-CoV (Grobben et al., 2021), supporting the hypothesis that conserved epitopes across coronaviruses can elicit immune recognition.

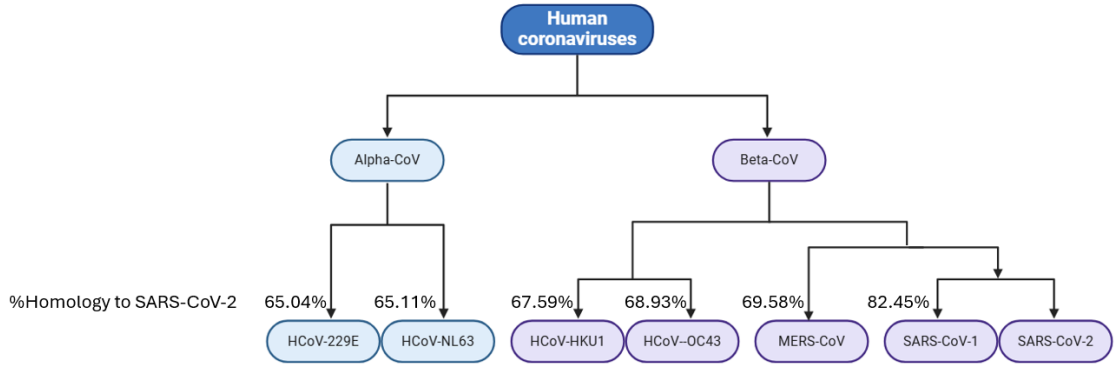

Figure 1-5 Lineage of human coronaviruses

Human coronaviruses are classified into alphacoronaviruses (HCoV-229E and HCoV-NL63) and betacoronaviruses (HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV-1 and SARS-CoV-2). Overall sequence homology between SARS-CoV-2 and other human coronaviruses ranges from approximately 65–82% (Kaur et al., 2021). *Created with Biorender.com.*

Multiple studies have detected SARS-CoV-2 reactive T-cells in samples collected before the COVID-19 pandemic, despite the use of highly sensitive T-cell assays, although non-specific binding cannot be fully excluded. SARS-CoV-2 unexposed individuals had ELISpot T-cell responses to SARS-CoV-2 nucleocapsid, NSP7 and NSP13 antigens (Le Bert et al., 2020). $CD4^+$ T-cells responses were detected against amino acids 101-120 of the SARS-CoV-2 nucleocapsid protein, a region that shares a high degree of homology with the nucleocapsid proteins of MERS, HCoV-OC43 and -HKU1 (Le Bert et al., 2020). $CD4^+$ T-

cells to SARS-CoV-2 NSP7 amino acid 26-40 and $CD8^+$ T-cells responses to NSP7 amino acid 36–50 were also detected in unexposed individuals, despite low sequence homology with other coronaviruses (Le Bert et al., 2020). Using activation-induced marker (AIM) assays, unexposed participants were found to have $CD4^+$ T-cells responses to SARS-CoV-2 spike, NSP14, NSP4, NSP6, whereas responses to nucleocapsid and membrane proteins were low (Grifoni et al., 2020), despite shared sequence homology with HKU1 (35.29% and 34.28%) and OC43 (38.74% and 35.20%) (Kaur et al., 2021).

The prevalence of cross-reactive T-cells measured varies depending on the assay platform and peptide pools used. Analyses using restricted epitope sets yielded higher estimates than those using larger peptide pools (Kundu et al., 2022), reflecting differences in assay sensitivity and specificity. Sample type also influences detection of cross-reactive T-cells. Only limited $CD8^+$ T-cells cross-reactivity was detected in peripheral blood and bronchoalveolar lavage (BAL) samples from unexposed individuals (Diniz et al., 2022; Grifoni et al., 2020). More memory $CD8^+$ T-cells were found in tonsils, a secondary lymphoid site, rather than in peripheral blood (Niessl et al., 2021). Some predicted $CD8^+$ T-cell epitopes that cross-react with SARS-CoV-2 are shared with other coronaviruses (Diniz et al., 2022; Lee et al., 2020) and with influenza virus (Schulien et al., 2021).

In addition to seasonal coronaviruses, other pathogens may provide cross-reactive antigens (Le Bert et al., 2020; C. Tan et al., 2021). Positive-strand ssRNA viruses, such as Togaviridae and Caliciviridae, share conserved regions within RNA-dependent RNA polymerase (RdRp) with SARS-CoV-2 (Ahmadi et al., 2021). Negative strand ssRNA paramyxoviruses fusion proteins also have sequence similarity to the SARS-CoV-2 S2 protein and to HCoV-HKU1 (Ahmadi et al., 2021).

Although Bacille Calmette-Guérin (BCG) vaccination, a live attenuated *Mycobacterium bovis* to prevent tuberculosis, does not providing cross-reactivity with SARS-CoV-2, it has been associated with the induction of trained immunity. Trained immunity is a characteristic of innate immune cells, especially myeloid cells, undergo epigenetic and metabolic reprogramming, which may amplify or dampen the subsequent non-specific immune response to heterologous pathogens or vaccines (Netea et al., 2020). Some countries implemented BCG vaccination had lower rate of SARS-CoV-2 infection, which has been the rationale of administering BCG as one of the tools during pandemics. However, a randomised clinical trial of BCG-Denmark vaccination in healthcare workers demonstrated no reduction in COVID-19 incidence at 6 months compared with placebo (Pittet et al., 2023) and the risk of symptomatic infection, in fact, increased at 12 months among BCG-vaccinated HCWs (Messina et al., 2024). As participants received BCG vaccination before COVID-19 vaccination, the authors suggested a potential negative effect to the induction of vaccine responses (Messina et al., 2024). Another randomised study in Denmark then showed reduced risk to SARS-CoV-2 infection over 12 months in participants <75 years and in those who received BCG vaccinations after COVID-19 vaccination (Madsen et al., 2024).

1.7.1.1 Impact of cross-reactive immune responses against SARS-CoV-2

The impact of pre-existing cross-reactive immune responses to SARS-CoV-2 had been reviewed elsewhere (Murray et al., 2023). Murray *et al*. concluded that cross-reactive T-cells against SARS-CoV-2 are associated with partial protection against adverse clinical outcomes, whereas the effects of cross-reactive antibodies are more variable (Murray et al., 2023). One plausible protective mechanism is the imprinting of T-cell help that enables rapid viral clearance, thus limiting progression to severe disease, a phenomenon previously

observed in influenza infection (Nelson & Sant, 2019). Supporting this, household contacts who remained PCR-negative despite repeated exposure to SARS-CoV-2 had high frequencies of cross-reactive memory T-cells, mainly targeting ORFs, N and M proteins (Kundu et al., 2022). In addition, Swadling *et al*. identified the viral replication-transcription complex, a highly conserved region, as the main target of pre-existing T-cells in highly exposed HCWs that remain seronegative. These responses were proposed to contribute mechanistically to abortive SARS-CoV-2 infection, characterised by viral control without seroconversion (Swadling et al., 2022). Pre-existing memory Tfh may also accelerate neutralising antibody production, while memory $CD4^+$ and $CD8^+$ T-cells could facilitate viral clearance in the lungs and upper respiratory tract (Lipsitch et al., 2020; Sette & Crotty, 2020). Furthermore, increased functional avidity of both T-cells and IgG responses to SARS-CoV-2 were also observed when pre-existing $CD4^+$ T-cells to S2 of spike were boosted during infection, potentially contributing to milder disease (Loyal et al., 2021).

In contrast, the impact of cross-reactive antibodies remains inconsistent across studies. Higher IgG titres to HCoVs have been associated with asymptomatic SARS-CoV-2 infection (Ortega et al., 2021) and recent exposure to HCoVs was associated with lower odds of intensive care unit (ICU) admission among hospitalised COVID-19 patients (Sagar et al., 2021). Conversely, another study did not find any relationship between HCoVs spike-specific antibody levels and disease severity in hospitalised patients (Anderson et al., 2021). Analysis of a larger and more severity-balanced cohort reported that antibodies to alphacoronavirus nucleocapsid proteins and to spike protein of HCoV-OC43 were associated with increased susceptibility to SARS-CoV-2 infection (Wratil et al., 2021). The discrepancies between studies likely reflect differences in cohort composition, case severity distribution as indicated by Wratil *et al*. Independent replication in additional cohorts stratified by disease severity will be required to confirm this finding.

Pre-existing cross-reactive immune responses may influence SARS-CoV-2 vaccine-induced immunity. In principle, cross-reactive T-cells could recognise conserved epitopes and contribute to early vaccine responses. However, a recent study using combined AIM+ and single cell transcriptomic sequencing, showed that pre-existing T-cells contributed minimally to vaccine-induced responses (Murray et al., 2025). mRNA vaccination recalled only a small number of pre-existing spike-specific T-cells, mainly the $CD8^+$ T-cells, whereas this was not observed following ChAdOx1 vaccine (Henze et al., 2023; Murray et al., 2025). Cross-reactive T-cells can also compete with naïve T-cells despite having lower TCR avidity, which reduce the proliferation of *de novo* T-cells to SARS-CoV-2 (Bacher et al., 2020; Dykema et al., 2021). The effect of pre-existing T-cells to human coronaviruses was reported as minimal in the generation of S-specific T-cell after vaccination (Saggau et al., 2022). Another study reported that pre-existing T-cells have also been associated with enhanced T-cell functional avidity following BNT162b2 vaccination (Loyal et al., 2021), suggesting that cross-reactive T-cells may promote vaccine-elicited responses.

The impact of pre-existing cross-reactive antibodies on SARS-CoV-2 vaccine responses remains uncertain. Detecting such effects in humans is challenging, as vaccination, particularly highly immunogenic mRNA platforms, induces high antibody titres in most individuals, limiting the ability to discern the influence of baseline HCoV immunity. In SARS-CoV-2 naïve individuals, no correlation was observed between baseline antibody levels to seasonal HCoVs and post-vaccination SARS-CoV-2 specific antibody responses (Lin et al., 2022; Moncunill et al., 2022). In contrast, among previously infected individuals, higher baseline IgG titres to the nucleocapsid protein of HCoV-HKU1 were negatively associated with subsequent spike-specific IgG responses to SARS-CoV-2 (Moncunill et al., 2022). This observation may be because prior SARS-CoV-2 infection increased antibody levels to HCoVs and SARS-CoV-2, potentially dampening further boosting after

vaccination. There is still a gap in understanding how pre-existing HCoV antibodies influence SARS-CoV-2 vaccine responses. Longitudinal study designs that assess within individual changes over time using fold-changes in antibody responses before and after vaccination are likely to be more informative than single time-point analyses. Moreover, assessing antibody avidity and functional properties may provide deeper insight into the quality and durability of vaccine-induced immunity.

1.7.2 Immunosenescence

Vaccine optimisation strategies must account for the aging of the immune system or immunosenescence. Remodelling of the immune system occurs over time due to cumulative damage from intrinsic processes (e.g., metabolic reactive oxygen species and telomere shortening) and extrinsic exposures (e.g., UV radiation, chemicals, pathogens) (Franceschi et al., 2007). These contribute to age-associated alterations in immune system, including low-grade inflammation and changes in lymphocytes compositions, which compromise responses to novel antigens. Thymic involution, where the thymus reduces in size and function over time, reduces the production of naïve T cells. Ageing in healthy individuals is marked by increased CXCL17, IL6, IL11, decline in naïve CD8 T-cells and increase of $CD27^-$ effector B-cells (Gong et al., 2025). Age has been associated with reduced vaccine responsiveness as reported in several studies (Collier et al., 2021; Costa et al., 2022; Palacios-Pedrero et al., 2022; Swanson et al., 2021). Elderly individuals with hypertension also showed lower antibody titres after the third mRNA vaccine dose (Ravussin et al., 2023), while being physically active along with normal BMI, HDL, glucose concentrations can improve the COVID-19 vaccination outcome, higher IgG to S, in elderly population (Kuijpers et al., 2023).

Changes in the immune system are shaped not only by chronological age but also by individual's physiological and functional state or biological age. Chronic infection, such as cytomegalovirus (CMV) infection, in individuals under 65 years can also alter immune composition, such as expansion of cytotoxic $CD8^+$ Tem, $CD4^+$ Tem, γδ T-cells and adaptive NK cells, changes that differ from those observed in elderly population (Gong et al., 2025). Persistent infections can induce the expansion of virus-specific T-cell populations, reduce the diversity of the naïve T-cell pools available to respond to new antigens and increased T-cell senescence, leading to weaker vaccine-induced T-cell responses(Kadambari et al., 2020).

Immune ageing due to age or chronic diseases is marked by the accumulation of senescent T-cells (Goronzy & Weyand, 2019; Pawelec, 2019). As somatic cells repeatedly divide, they eventually reach a replicative limit, known as Hayflick limit, at which point cell-cycle arrest or replicative senescence occurs (Hayflick & Moorhead, 1961). Telomere length shortens in CD4+ T-cells, CD8+ T-cells and B-cells with age (Son et al., 2000). Telomerase activity depends on CD28 signalling and loss of CD28 expression is a marker of senescent T-cells (Valenzuela & Effros, 2002). CD28 loss on $CD8^+$ T-cells is associated with reduced IFN-γ production but increased pro-inflammatory TNF, which plays a key role in NK cells recruitment (Effros et al., 2005). $CD57^+$ is a marker of terminal differentiation and reduced proliferative ability, and its expression on $CD8^+$ T-cells, $CD4^+$ T-cells and NK cells, has been observed in chronic infections, such as CMV and HIV (Brenchley et al., 2003; Wertheimer et al., 2014). Typing of $CD8^+CD27^-CD28^-CD57^+$ and KLRG-1^+ can define senescent-cells (Pawelec, 2019).

IFN-γ responses following two doses of BNT162b2 mRNA vaccine were inversely correlated with the expression of age-dependent senescent markers on $CD8^+$ T-cells

(Palacios-Pedrero et al., 2022). In contrast, CMV seropositivity did not impair the induction of anti-spike IgG and NAb and $CD4^+$ memory T-cell following vaccination with SARS-CoV-2 mRNA vaccination (Breznik et al., 2022). However, senescent T-cells, $CD28^-CD57^+CD4^+$ and $CD8^+$ T-cells, were higher in elderly seropositive with CMV than those seronegative who received mRNA vaccination (Breznik et al., 2022). Hence, heterogeneity in vaccine responses is unlikely to be explained by a single demographic or immunological marker, rather it reflects the combined influence of multiple features of immune ageing.

Emerging evidence has suggested that repeated mRNA booster vaccination may be associated with exhaustion phenotypes among older individuals or those with chronic inflammation. Older adults exhibited increased $PD1^+LAG3^+TIM3^-$ spike-specific CD8+ T-cells after their third and fourth doses of vaccine compared with post second dose, but no changes in T-cell polyfunctionality (Benoit et al., 2025). However, the longitudinal analysis of healthy infection-naïve or asymptomatic individuals showed no increase of exhausted $CD8^+$ T-cell clusters following second, third and fourth mRNA vaccine dose (da Silva Antunes et al., 2025). Cancer patients with decreased IFN-γ production after their third dose compared with after their second dose of mRNA 1273 vaccine had a higher expression of $PD1^+$ and $PD1^+CD57^+CD8^+$ T-cells than those with boosted IFN-γ production (Benitez Fuentes et al., 2022). No changes of exhaustion markers observed in $CD4^+$ T-cells in older individuals and younger individuals following multiple vaccine doses (Benoit et al., 2025).

1.8 Aim and Objectives

Vaccine-induced immune responses depend on both vaccine immunogenicity and host immunocompetence. Different vaccine platforms, such as mRNA, viral vector, and inactivated whole-virus vaccines, elicit distinct immune responses due to their differing antigenic compositions, yet comparative data across populations remain limited. Prior

exposure to seasonal human coronaviruses may imprint the immune system in ways that further modulate responses to SARS-CoV-2 vaccination, and features of immune ageing, driven by age and persistent infection, may alter vaccine-induced immunity. Addressing these knowledge gaps is important both for explaining the influencing factors of vaccine-induced immune responses related to the vaccine and the host, which can inform future vaccine design, and for supporting vaccination strategies that account for heterogeneity in immune responsiveness at the population-level.

Research Questions

- What are the humoral and cellular immune profiles of vaccinated individuals without known history of SARS-CoV-2 infection?
- What proteomic and demographic factors are associated with T-cell responses following SARS-CoV-2 vaccination in immunocompetent individuals and what immunophenotypic characteristics distinguish T-cell responders from non-responders?
- How do humoral and cellular immune responses differ across SARS-CoV-2 vaccine platforms and how does baseline immunity influence these responses?

My thesis aims to assess humoral and cellular immune responses following diverse SARS-CoV-2 vaccination in real-world populations. To achieve this aim, humoral and cellular responses to SARS-CoV-2 and seasonal human coronaviruses were assessed, alongside demographic and baseline immunological factors associated with vaccine responsiveness in cohorts from the UK and Indonesia.

I hypothesise that pre-existing T-cell and humoral immune responses arising from prior SARS-CoV-2 exposure may contribute to enhanced vaccine-induced immunity, whereas chronic inflammation and persistent infections are associated with reduced vaccine responsiveness. SARS-CoV-2 vaccine platforms differ in the magnitude and breadth of

immune responses, with mRNA and viral vector vaccines eliciting strong spike-specific immunity, while inactivated whole-virus vaccines induce broader responses that include non-spike antigens.

Objectives

1. To characterise spike- and non-spike-specific antibodies and T-cell responses from vaccinated healthcare workers without documented SARS-CoV-2 infection.
2. To identify proteomic and demographic predictors of T-cell responsiveness following two doses of mRNA SARS-CoV-2 vaccination, and to compare the immunophenotypic profiles of T-cell vaccine response and non-response groups at one-month post-vaccination.
3. To compare humoral and cellular immune responses across inactivated, mRNA and viral vector vaccine platforms, and to evaluate the influence of prior exposure to SARS-CoV-2 on vaccine-induced anti-spike IgG responses.

2 Materials and methods

2.1 Materials

Commercial and in-house materials used in my research project are listed in the following tables (Table 2-1 and Table 2-2).

Table 2-1 List of commercially available materials used in this thesis

Item	Catalogue Number	Company
1-Step™ NBT/BCIP substrate	34042	Thermo Scientific
1x Phosphate buffered saline (PBS) pH 7.4 1x	10010-015	Gibco™
4% formaldehyde, pH 6.9	1.00496.0700	Sigma-Aldrich
96-roundbottom plate	83.3925	SARSTEDT
96-well Multiscreen-I ELISpot plates	MAIPS4510	Millipore
Anti CD28 1mg/mL	340975	BD Biosciences
Anti CD49d 1 mg/mL	340976	BD Biosciences
Anti-human CD14 APC-Fire750	301854	Biolegend
Anti-human CD14 PerCP	325632	Biolegend
Anti-human CD19 FITC	302206	Biolegend
Anti-human CD3 BV510	300448	Biolegend
Anti-human CD3 FITC	300440	Biolegend
Anti-human CD3 PerCP	300428	Biolegend
Anti-human CD4 APC	300514	Biolegend
Anti-human CD4 FITC	300506	Biolegend
Anti-human CD56 BV421	318328	Biolegend
Anti-human CD8a BV501	301048	Biolegend
Anti-human CD8a PE/Cyanine7	301012	Biolegend
Anti-human IFN-γ PE	502509	Biolegend
Anti-human IFN-γ mAb 7-B6-1-biotin, biotinylated	3420-6-1000	Mabtech
Anti-human IFN-γ monoclonal antibody (mAb) 1-D1K	3420-3-1000	Mabtech, AB, Sweden
Anti-human IL2 PE-Cy7	25-7029-42	eBioscience
Anti-human TNFα APC	502912	Biolegend
ArC Amine Reactive Compensation Bead Kit	A10346	invitrogen
BD Comp Beads	51-90-9001229	BD Biosciences
BD Comp Beads Anti-Rat and Anti-Hamster	51-90-9000949	BD Biosciences
BD Comp Beads Negative Control	51-90-9001291	BD Biosciences

BD Fixation/permeabilization kit (Cytofix/Cytoperm solution and 10x Perm/Wash buffer)	554714	BD Biosciences
Benzonase nuclease, purity >99%	70664-3KUN	Merck Millipore
Brefeldin A 1000x	420601	Biolegend
Caspase 3 PE	51-68655X	BD Biosciences
CEF	PX-CEF-G and PX-CEFT-G	Proimmune or Genescript
Cell activation cocktail without BFA (contains PMA/Ionomycin) 500x	423301	Biolegend
Cell counting chamber slide	100078809	Invitrogen
Cell staining buffer	42020	Biolegend
CellTrace Violet (CTV)	C34557	Invitrogen
Centrifuge	5810 R	eppendorf
Countess 3 Automated Cell Counter	AMQAX2000	Invitrogen
Dimethyl sulfoxide (DMSO)	D2650	Sigma
IgG enzyme-linked immunosorbent assay (ELISA) kits: Cytomegalovirus (CMV)	EI 2570-9601 G	EUROIMMUN
IgG ELISA kits: Human Src Kinase Associated Phosphoprotein 2 (SKAP2)	abx383214	Abbexa
IgG ELISA kits: human matrix metalloproteinase 1 (MMP1)	abx050157	Abbexa
IgG ELISA kits: Human interleukin-1 receptor-like 2(IL1RL2)	abx385042	Abbexa
IgG ELISA kits: Human collagen type IX alpha 1 (COL9A1)	abx507798	Abbexa
IgG ELISA kits: Human eukaryotic translation initiation factor 5A-1 (EIF5A)	abx387113	Abbexa
FcR Blocking Reagent human	130-059-901	Miltenyi Biotec
Human Ab serum	H3667	Sigma Aldrich
ImmunoSpot®Series 6 alfa ELISpot Analyzer	s/n S6ENTRY-01-1114	C.T.L. Europe GmbH
Incubator	C170i	Eppendorf/CellXpert
L/D Near Infra-Red	L34976	Invitrogen
Leucosep tubes	227290	Greiner Bio-one
L-Glutamine	G7513	Sigma Aldrich
Lymphoprep™	04-03-9391/02	Serumwerk Bernburg AG
MACSQuant Analyzer 10 Flow Cytometer		Miltenyi Biotec
MSD Multi-Spot Assay kit, Coronavirus Plate 2 Multispot 96-well, 10 spot plate	N05368A-1	MesoScale Discovery
Penicillin/Streptomycin	P0781	Sigma Aldrich
Phosphate buffered saline	P4417	Sigma Aldrich
Phytohemagglutinin L (PHA-L)	11249738001	Sigma Aldrich

Protease Inhibitor Cocktail set I	539131	Calbiochem
RPMI-1640 Medium with Sodium bicarbonate	R0883	Sigma Aldrich
Streptavidin alkaline phosphatase	3310-10-1000	Mabtech
Trypan blue stain 0.4%	T10282	Invitrogen
Tween® 20	P2287	Sigma

Table 2-2 List of in-house made materials used in this thesis

Item	Components
Coating buffer for ELISpot	Carbonate-bicarbonate buffer capsule in 100 ml deionized water then filtered (0.2um)
Elution buffer	0.5g of 1% BSA dissolved in 49.5 mL PBS + 1x Protease Inhibitor Cocktail set I
Freezing mix	foetal bovine serum with 20% of DMSO
Milli-Q water (MQ-H20)	Autoclaved MQ-H2O
PBS- 0.05% Tween 20	PBS 1x and 0.05% of Tween-20
Phosphate Buffered Saline (PBS) 1x	1 tablet + 200 mL of MiliQ water then autoclaved
R0	RPMI-1640 Medium with Sodium bicarbonate, with 1 mM Penicilin/Streptomycin and 2mM L-Glutamine
R10	RPMI-1640 Medium with Sodium bicarbonate, with 1 mM Penicilin/Streptomycin and 2mM L-Glutamine and 10% fetal bovine serum
Rab10	RPMI-1640 Medium with Sodium bicarbonate, with 1 mM Penicilin/Streptomycin and 2mM L-Glutamine and 10% human Ab serum

2.2 Study populations

The list of ethical approval information of research studies included in this thesis is shown in Supplementary Table 1.

2.2.1 UK healthcare worker cohort – Chapter 3

Plasma, serum, nasal fluid and peripheral blood mononuclear cells (PBMC) samples were obtained from vaccinated individuals who self-reported no known COVID-19 infection (NKI group) from the "*Protective Immunity from T-cells to COVID-19 in Health workers*" (PITCH) (Research Ethics Committee (REC) ref.: 21/YH/0206) and the "*Vaccine Immunity, Breakthrough & Reinfection - Antibodies & T-cells"* (VIBRANT) (REC ref.: 21/HRA/5433) studies. Participants were healthcare workers (HCWs) recruited across the UK between July 2020 and April 2023. Samples from vaccinated individuals enrolled in the PITCH study in Oxford who had polymerase chain reaction (PCR) or lateral flow test (LFT) confirmed SARS-CoV-2 breakthrough infection 15-30 days prior to sampling were included as the documented infection (DI) group. Additional samples were obtained from unexposed individuals with no known SARS-CoV-2 infection and no vaccines (unexposed-UE group), collected in early 2020 as part of the COVID-19 Convalescent immunity (COCO) study in Birmingham, UK (REC ref.: 20/HRA/1817). All UE participants tested negative for anti-SARS-CoV-2 S glycoprotein IgG/A/M antibodies by ELISA (Faustini et al., 2021).

2.2.2 Healthy and immunocompromised patient cohorts - Chapter 4

Plasma and serum samples from infection-naïve healthcare workers were collected through studies conducted in Birmingham (COCO study: London – Camden & King's Cross Research Ethics Committee (ref. 20/HRA/1817)), Liverpool ("Human immune responses to acute virus infections" - North West - Liverpool Central Research Ethics Committee Study

(ref. 16/NW/0170)), Newcastle (ref: 20/SC/023), Oxford ("*Protective Immunity from T-cells to COVID-19 in Health workers*" (PITCH) (ref. 21/YH/0206) and Sheffield (Observational Biobanking study STHObs (ref 18/YH/0441). Samples were collected either at baseline or prior to the second dose of COVID-19 vaccination between April 2020 and April 2021. All participants had no documented history of SARS-CoV-2 infection, as determined by negative PCR or LFT results. Data on IFN-γ secreting T cells at V2+28 days timepoint were available from previous studies (Angyal et al., 2022; Moore et al., 2023; Neale et al., 2023; Payne et al., 2021). A T-cell responder was defined as an individual having >50 spot forming units per million PBMCs to SARS-CoV-2 spike, as measured by IFN-γ ELISpot. This threshold was determined based on background responses observed in negative control wells.

PBMC samples from PITCH sites available at the V2+28 days timepoint were used for immunophenotyping analysis. In addition, PBMC samples from immunocompromised individuals recruited through the COVID-19 in patients with antibody deficiency (COV-AD) study (Shields et al., 2022) were included. The COV-AD study received ethical approval from the London - Dulwich Research Ethics Committee (REC reference: 21/LO/0162). Patients with primary or secondary antibody deficiency were recruited across the UK from March 2021 onwards. Participants were enrolled if they were receiving immunoglobulin replacement therapy, or had a serum IgG concentration <4g/L and were receiving antibiotic prophylaxis to prevent infections. Participants were followed according to the national SARS-CoV-2 vaccination schedule. PBMC samples included in the analysis were collected following receipt of the third COVID-19 vaccine dose.

2.2.3 Indonesian community cohort- Chapter 5

"*Evaluation of adaptive immunity following inactivated whole-virus vaccination in Indonesian populations*" (EVAIC) study is a secondary analysis study of the INVITE study - an observational study in Jakarta, Indonesia (December 2021-present): "*Real-world assessment of the immunogenicity and effectiveness of COVID-19 vaccines in Indonesia*: Longitudinal observations of vaccine-induced immune responses, adverse reactions and SARS-CoV-2 breakthrough infections". Paired samples before first dose and one month after second dose of COVID-19 vaccines were selected from the INVITE biorepository. PBMC and serum were collected from healthy participants receiving their COVID-19 vaccinations at primary health care centres, Puskesmas Ciracas and Puskesmas Cakung, or Carolus hospital, in Jakarta, Indonesia. Participants received either homologous doses of inactivated vaccine Sinovac/CoronaVac, viral vector vaccine Oxford/AstraZeneca or mRNA vaccine Pfizer/BioNtech. Samples were collected between November 2021 and August 2022. The EVAIC study received ethical approval from Faculty of Medicine, Universitas Indonesia (KET-418/UN2.F1/ETIK/PPM.00.02/2023) and Oxford Tropical Research Ethics Committee (OxTREC) ref 544-23.

The process of getting the approval to ship samples from Indonesia to Oxford was protracted and challenging as listed in the Table 2-3.

Table 2-3 Timeline for sample shipment from Indonesia to Oxford

Date	Material Transfer Agreement (MTA) Application Process
May-Aug 22	Study document preparation for EC submission
04 Nov 22	EC submission of EVAIC study as a sub-study
10 Jan 23	EC rejection as a sub-study to INVITE study and request for a re-submission as a standalone study
07 Mar 23	EC re-submission of as a standalone study EVAIC study
10 Apr 23	EC approval Faculty of Medicine, Universitas Indonesia EC until 09 Apr 24
08 Jun 23	Submission to MTA committee at MOH
28 Jul 23	Submission to OXTREC
4 Oct 23	OxTREC approval
12 Jan 24	Extension approval of local EC until 09 Apr 25
31 May 24	Interview by Indonesian Ministry of Health. Outcome: to change EVAIC PI to INVITE PI
11 Sep 24	Submission to local EC of protocol amendment v1.1
30 Sep 24	Local EC approval of protocol amendment v1.1
15 Nov 24	OxTREC approval of protocol amendment v1.1
07 Feb 25	Finalisation of agreement between OUCRU Indonesia and Universitas Indonesia
17 Feb 25	Extension approval of local EC until 09 Apr 26
11 Mar 25	MTA Approval from Indonesian Ministry of Health
13 Mar 25	Finalisation of institutional study agreement between Oxford and Universitas Indonesia
22 Apr 25	Sample arrival in Oxford

2.3 Plasma, serum and PBMC isolation and storage

Blood was collected in serum separator tubes for serum and in Ethylenediaminetetraacetic acid (EDTA) tubes for plasma and PBMC isolation. Serum was separated from whole blood by centrifuging the serum separator tube at 845xg for 10 mins. To isolate plasma and PBMC, Lymphoprep^{TM} (04-03-9391/02, Serumwerk Bernburg AG) (07861, StemCell Technologies) was added into Leucosep tubes (227290, Greiner Bio-one). The tubes were spun in a centrifuge machine at 1000xg for 1 min, so the lymphoprep would sit below the

filter disc. Whole blood was poured into the tubes and centrifuge at 1000xg for 15 mins at room temperature without break. Plasma was taken and placed into another tube for storage at -70 ^{o}C. PBMC were collected using a Pasteur pipette and place into a new tube. PBMC were washed with R0 and centrifuged at 845xg for 10 mins twice. The supernatant was discarded, and the pellet was resuspended with cold freezing mix (foetal bovine serum with 20% of dimethyl sulfoxide (DMSO) (D2650, Sigma). Cells were then stored at -80 ^{o}C before being cryopreserved in liquid nitrogen (LN2).

2.4 Nasal fluid collection and storage

Mucosal lining fluid from the nose was collected using NasosorptionTM FX·I device (Hunt Developments UK Ltd), a synthetic absorptive matrix (SAM) strip. SAM strip was inserted in one nostril and placed against the inferior turbinate for 60 seconds. Samples were refrigerated for a maximum of 2 hours and transferred to -80 °C freezer. Prior to analysis, the SAM strips were thawed on ice for 30 mins. Elution buffer (500uL) was made by 0.5g of 1% BSA dissolved in 49.5 mL PBS + 1x Protease Inhibitor Cocktail Set I. Elution buffer was added and the samples were incubated for 15 mins on ice. Samples were centrifuged at 16,000xg for 15 mins at 4°C. Eluted samples were stored at -80 °C.

2.5 MesoScale Discovery (MSD) assay

Antibody data were generated with Melissa Govender at the laboratory of Miles Carroll, University of Oxford. Binding antibodies and ACE2 inhibition antibodies were measured using MSD assay per manufacturer's instructions. Briefly, plates pre-coated with antigens of interest (Coronavirus Plate 2: CoV-2 S, CoV-2 N, CoV-1 S, CoV-2 NTD, NL63 S, HKU1 S, OC43 S, 229E S, CoV-2 RBD) were blocked using blocker solution for 30 mins. Plates were then washed three times using a washing buffer. Samples, calibrators, and controls

were added to wells and incubated for 2 hours. Plates were washed three times again before adding detection antibody and incubated for 1 hour. In case of ACE2 neutralisation assay, plates were not washed instead detection ACE2 was added and incubated for 1 hour. Plates were washed again before adding a reading buffer prior and reading on an MSD instrument.

Seropositivity cut-offs for IgG in plasma/serum were set based on mean + 3SD measured in pre-pandemic SARS-CoV-2 negative Scottish donors (n=64) as previously calculated in another study (Barnes et al., 2023): 1120.59 (SARS-CoV-2 S), 1865.64 (SARS-CoV-2 RBD), 32.55 (SARS-CoV-2 NTD), 999.58 (SARS-CoV-1 S) and 2957.25 (SARS-CoV-2 N). Seropositivity cut-offs for IgA in plasma/serum were: 505.37 (SARS-CoV-2 S), 999.22 (SARS-CoV-2 RBD), 565.75 (SARS-CoV-2 NTD), 382.20 (SARS-CoV-1 S) and 1358.69 (SARS-CoV-2 N).

Seropositivity cut-offs for nasal antibodies were measured in pre-pandemic samples (n=4-5) (Figure 2-1, Table 2-4). Seropositivity cut-offs for nasal IgG: 1.70 (SARS-CoV-2 S), 4.08 (SARS-CoV-2 RBD), 0.04 (SARS-CoV-2 NTD), 1.04 (SARS-CoV-1 S) and 5.87 (SARS-CoV-2 N). Seropositivity cut-offs for nasal IgA were: 10.46 (SARS-CoV-2 S), 13.11 (SARS-CoV-2 RBD), 8.25 (SARS-CoV-2 NTD), 3.32 (SARS-CoV-1 S) and 10.41 (SARS-CoV-2 N). No seropositivity cut-off values were available for antibodies specific to human seasonal coronaviruses or for ACE2 percentage inhibition.

Table 2-4 Nasal antibodies to SARS-CoV-2 measured in pre-pandemic samples used to calculate assay cut-offs

Nasal IgG		Cov-2 RBD	Cov-2N	Cov-2S	CoV-2 NTD
	Control SAM 4	1.57	2.70	1.00	0.03
	Control SAM 7	1.32	3.92	0.85	0.02
	Control SAM 9	1.53	1.69	0.15	0.02
	Control SAM 14	0.22		0.27	0.01
	Control SAM 15	2.68	1.36	0.68	0.02
	Average	1.46	2.42	0.59	0.02
	SD	0.87	1.15	0.37	0.01
	Average + 3SD	4.08	5.87	1.70	0.04

Nasal IgA		Cov-2 RBD	Cov-2N	Cov-2S	CoV-2 NTD
	Control SAM 4	9.03	5.43	5.64	5.56
	Control SAM 7	4.99	8.13	1.47	1.53
	Control SAM 9	7.58	7.32	6.57	4.01
	Control SAM 14	6.12		3.86	2.81
	Control SAM 15	3.00	7.15	2.26	1.55
	Average	6.14	7.01	3.96	3.09
	SD	2.32	1.13	2.17	1.72
	Average +3SD	13.11	10.41	10.46	8.25

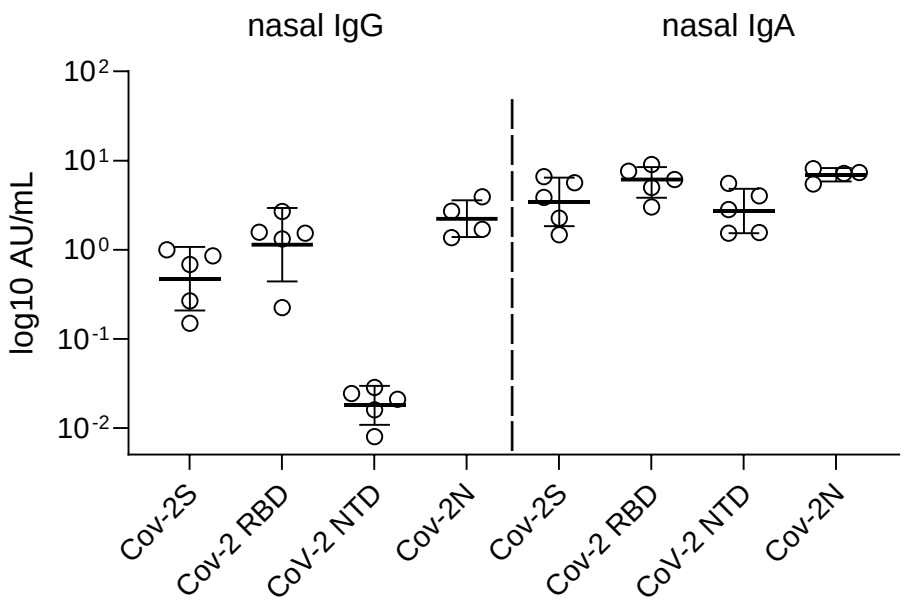

Figure 2-1 Nasal antibodies to SARS-CoV-2 measured in pre-pandemic samples

Nasal IgG and IgA responses were measured using the MesoScale Discovery (MSD) platform. Antibodies were measured against SARS-CoV-2 spike (S), SARS-CoV-2 receptor binding domain (RBD), SARS-CoV-2 N-terminal domain (NTD), SARS-CoV-2 nucleocapsid (N). Each data point represents an individual sample. Error bars represent the mean ± one standard deviation.

2.6 PBMC thawing

Frozen PBMCs were thawed in a 37 ºC water bath. Once nearly thawed, cells were transferred into 10 mL of R10 medium. Cells were centrifuged, the supernatant discarded and the pallet washed with 5 mL of R10 before centrifugation. Cell pellets were resuspended in R10, with or without Benzonase nuclease, purity >99% (70664-3KUN, Merck Millipore), at a dilution of 1:5000. Cells were incubated for at least 30 mins prior to counting and resuspended at the concentration needed for the assays. To count cell number, 10 µL cells in R10 were mixed with 10 µL of trypan blue stain 0.4% (T10282, Invitrogen). 10 µL of

stained cell suspension was loaded onto a cell counting chamber slide (100078809, Invitrogen) and cells were counted using an ImmunoSpot® Analyzer.

2.7 IFN-γ ELISPOT

96-well Multiscreen-I ELISpot plates were coated with 50 µL of 10 µg/ml anti-human IFN-γ monoclonal antibody (mAb) 1-D1K for 3 hrs at room temperature or 48 hrs at 4 ^{o}C fridge. Plates were then washed with 100 µL of R0 twice before blocking the plates with Rab10 for one hour. PBMC were added in duplicates at 200,000 cells/50 uL. 50 µL of each peptide pool (4µg/mL) were then added to stimulate the cells. Peptides used were overlapping peptides of S1, S2, M+N, NSP3b, NSP7-11, NSP12a, NSP12b, NSP13, and ORF3 regions. dimethyl sulfoxide (DMSO) was added in negative control wells and cytomegalovirus (CMV), Epstein-Barr virus (EBV), and influenza virus (CEF) and Concanavalin A (ConA) as stimulants in positive control wells. Cells were incubated at 37 ^{o}C, 5% CO2, 95% humidity for 16-18 hours. Next, plates were washed with phosphate buffered saline (PBS)-Tween® 6 times before adding the secondary antibody, 50 µL of 1 µg/ml anti-human IFN-γ mAb 7-B6-1-biotin, biotinylated for 2 hours. Plates were washed again before adding the tertiary antibody, 50 µL of 1 µg/ml streptavidin alkaline phosphatase for 1-2 h. To develop the plates, 50 µL of 1-Step^{TM} NBT/BCIP substrate were then added and incubated for 5 minutes before washing the plates under running distilled water. After the plates dry, plates were then read using AID ELISpot Reader (v.4.0). For each sample, responses in the negative wells that were higher than 50 SFU/10^6 cells were excluded. Samples with low responses, <200 SFU/10^6 cells, in the positive control wells ConA were also excluded from further analysis. The response was then calculated by subtracting the mean value measured in negative controls (duplicate) from the test wells and expressed as spot-forming units

(SFUs)/10^6 PBMCs. The positivity cut-off of the assay was set at 2 SD above the mean background value.

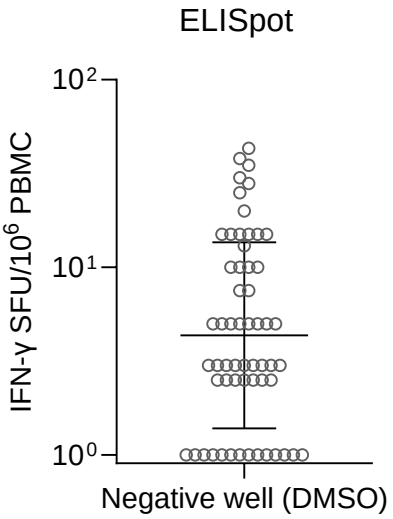

Figure 2-2 Background ELISpot responses in negative control (DMSO) wells

The mean number of spot-forming units (SFU) in duplicate negative control (DMSO) wells (n=59) is shown. Each datapoint represents the mean SFU count from duplicate negative wells for an individual sample. Error bars indicate the overall mean ± one standard deviation (SD). The mean background response was 7.7 SFU/10^6 PBMCs with an SD of 10.1. The seropositivity cut-off for the ELISpot assay was defined as the mean+2SD, corresponding to 27.9 SFU/10^6 PBMCs.

2.8 T-cell proliferation assay

PBMCs were washed with 1x PBS pH 7.4 1x (10010-015, Gibco™) twice. Cells were then stained with CellTrace Violet (CTV) (C34557, Invitrogen) at a final concentration of 2.5 µM and incubated in the dark for 10 mins at room temperature. The staining was stopped by adding cold fetal bovine serum (FBS) and kept in the 4ºC fridge for 5 mins. Cells were spun, supernatants were removed and resuspended in Rab10 (RPMI-1640 Medium with Sodium bicarbonate (R0883, Sigma Aldrich), with 1 mM Penicilin/Streptomycin (P0781, Sigma

Aldrich) and 2mM L-Glutamine (G7513, Sigma Aldrich) and 10% human Ab serum (H3667, Sigma Aldrich)). Cells were incubated at 37 ^{o}C, 5% CO2, 95% humidity for 5 mins, before plating. 250,000 cells/100 uL/well were added onto 96-round bottom plate (83.3925, SARSTEDT) in duplicates and were stimulated for 7 days with SARS-CoV-2 peptides (overlapping pools covering S1, S2, M, N, NSP3b, NSP7-11, NSP12a, NSP12b, NSP13, and ORF3) at concentration 2µg/mL. Additionally, negative control wells were added with DMSO and positive wells were stimulated using CEF and phytohemagglutinin L (PHA-L) (11249738001, Sigma Aldrich). Cells were incubated at 37 ^{o}C, 5% CO2, 95% humidity. On day 4, cells were spun in a centrifuge and half of the supernatant was removed and new Rab10 were added to each well. On Day 7, cells were washed in 1xPBS once, before staining with anti-human CD3 FITC (300440, Biolegend), 1:200 anti-human CD4 APC (300514, Biolegend), 1:200 anti-human CD8a PE/Cyanine7 (301012, Biolegend) and 1:1000 L/D Near Infra-Red (L34976, Invitrogen) (Supplementary Table 2). 1xPBS were added to wash the cells. Cells were then fixed using 100µL 4% formaldehyde, pH 6.9 (1.00496.0700, Sigma-Aldrich) and incubated at 4 ^{o}C for 10 mins, before adding another 100µL of PBS. Cells were spun again and resuspended in 100µL 1xPBS for reading at MACSQuant Analyzer 10 Flow Cytometer (Miltenyi Biotec). Data were excluded from the analysis if lymphocytes and live CD3+ cell counts were <1000 and the proliferation percentage of CD4+ and CD8+ T-cells when stimulated with DMSO was <2%.

2.9 Olink protein marker measurement

The immune system is composed of a network of cells that coordinate immune responses via cytokine-mediated signalling and direct cell-cell interactions. Proteomics contributes to system-level profiling of vaccine immune responses by detecting proteins involved in signalling pathways providing mechanistic insight into innate and adaptive immune

activation which may improve to understand variability in vaccine responses (Chu et al., 2021). Proteomic profiling has been previously used to characterise immune responses following SARS-CoV-2 vaccine responses and infection (Hickey et al., 2025; Hu et al., 2024; Hufnagel et al., 2023; Hwangbo et al., 2022; Patel et al., 2021; Shen et al., 2020; Su et al., 2023; Williams et al., 2025).

Proteins (n=364) in serum and plasma were measured using the Olink® Explore 384 platform (inflammation panel). This panel is composed of proteins related to immunological and inflammatory disease biology. This proximity extension assay technology recognises each target protein using pairs of antibodies labelled with DNA oligonucleotides, thereby enhancing sensitivity and specificity. The DNA was then amplified by PCR and libraries are sequenced using next-generation sequencing (NGS) on Illumina's NovaSeq 6000. Protein abundance was reported as NPX (Normalised Protein eXpression) values, which are normalised and presented on log2-transformed protein expression values. Laboratory work, including library preparation and sequencing, was performed by the Multiomics Technology Platforms team at the Centre for Human Genetics, University of Oxford.

2.10 Anti-CMV ELISA

IgG to cytomegalovirus (CMV) was measured using enzyme-linked immunosorbent assay (ELISA) kits (EI 2570-9601 G, EUROIMMUN). Serum or plasma samples were diluted at 1:101 and added to microplates pre-coated with CMV antigen. Samples were measured in duplicate. Positive and negative controls and calibrators at three concentrations (200 RU/ml, 20 RU/ml and 2 RU/ml human IgG) were included on each plate. Plates were incubated at room temperature (RT) for 30 minutes, washed three times with 300 µL 1x wash buffer with 30-60 seconds intervals, then incubated with peroxidase-labelled anti-human IgG (rabbit) for 30 mins at RT. After washing, 100 µL of TMB/H2O2 substrate was added for 15 mins,

followed by 0.5 M sulphuric acid to stop the reaction. Plates were read on a FLUOstar Omega 0415F0005A (BMG Labtech) at 450 nm wavelength with a 620 nm reference. Each measurement at 450 nm was subtracted from the measurement at 620 nm. Semiquantitative results were calculated as the ratio of sample measurement to that of the 20 RU/mL calibrator and reported as: negative (ratio <0.8), borderline (ratio ≥0.8 to <1.1.) and positive (ratio ≥1.1).

2.11 Sandwich ELISA

To independently quantify protein markers identified by Olink proteomic profiling, selected proteins in serum or plasma were measured using sandwich ELISA kits: human Src Kinase Associated Phosphoprotein 2 (SKAP2) (cat no. abx383214, Abbexa), human matrix metalloproteinase 1 (MMP-1) (cat no. abx050157, Abbexa), human interleukin-1 receptor-like 2(IL1RL2) (cat no. abx385042, Abbexa), human collagen type IX alpha 1 (COL9A1) (cat no. abx507798, Abbexa), and human eukaryotic translation initiation factor 5A-1 (eIF5A) (cat no. abx387113, Abbexa). Assays were performed according to the manufacturer's instructions. Briefly, samples were diluted according to the manufacturer's recommendation: 1:100 (SKAP2), 1:10 (MMP-1), undiluted (IL1RL2, COL9A1, eIF5A), added to pre-coated plates in duplicates. Plates were incubated for 2 hours at 37^0C. After incubation, plates were washed, then incubated with biotin-conjugated reagent for 1 hour at 37 ^{o}C. Plates were washed 3x using 300µL of 1x wash buffer, with 1-2 soaking times between washes. HRP-conjugated reagent was then added to all the wells and plates were incubated for another hour at 37 ^{o}C. Solutions were discarded and plates were washed for five times using wash buffer to remove unbound conjugates 90 µL TMB substrate was added for 10-20 minutes to measure the enzymatic reaction. The stop solution was added and the intensity of colour was measured using a microplate reader GloMax® Explorer (GM3500,

Promega). The plate reader was set at 450 nm with a 560 nm reference. Each reading at 450 nm was subtracted from the measurement at 560 nm. The average reading of duplicate sample wells was then subtracted from the average of blank control reading. Standard curves were generated using a four-parameter logistic (4PL) model in PRISM. Unknown concentrations were extrapolated from the curve and multiplied by the dilution factor where applicable to obtain the final analyte concentrations for each biological sample.

2.12 Immunophenotyping

High-dimensional flow cytometry was performed using the 77-marker Immune Memory and Mechanisms of Protection from Vaccines (IMMPROVE) consortium panel (The IMMPROVE consortium) (Supplementary Table 3). Frozen PBMCs were thawed, counted, and prepared into 96-well plates (11897403, Fisher Scientific) before surface staining with viability dye and antibody master mix (Supplementary Table 4). Following washing, cells were fixed and permeabilized (554714, BD), blocked using blocking mix (Supplementary Table 5), then incubated overnight with intracellular staining antibodies (Supplementary Table 6). After final washes and buffer equilibration, samples were filtered to remove debris and acquired on the a 5-laser Sony ID7000 spectral flow cytometer. The laboratory work for this part of the study and the spectral unmixing were performed by Oliver Burton in Adrian Liston's Lab at the University of Cambridge.

2.13 Extracellular staining flow cytometry

Cells were resuspended in 1xPBS and were plated to 96-round bottom plates containing 200,000 cells/well. Antibody mixture made up of 1:100 anti-human CD3 BV510, 1:200 anti-human CD4 APC, 1:100 anti-human CD8a PE-Vio770, 1:50 CD56 BV421, 1:50 CD69 PE, 1:30 HLA-DR FITC, 1:200 CD14 APC-Fire750 and 1:1000 L/D Near Infra-Red

(Supplementary Table 7) was prepared in PBS and FcR blocking reagent (1:10 dilution). Cells were stained for 20 mins at 4 ^{o}C in the dark. Cells were washed twice in PBS by centrifuging the plate at 1800 rpm for 3 mins and the supernatant was discarded after each wash. Cells were resuspended in PBS for acquisition on MACS Quant.

2.14 Intracellular staining flow cytometry

PBMCs were resuspended at a concentration of $1x10^6$ cells/100 µL in R10 medium. Peptide stimulants were prepared at 8µg/mL and co-stimulants anti-CD28 and anti-CD49d were prepared at 4µg/mL in R10. Peptides and co-stimulants were added to 96-round bottom plates (83.3925, SARSTEDT) at 50µL per well and incubated at 37 ^{o}C for at least 1 hour. PBMCs were then added to the wells and cells were stimulated for 1 hour at 37 ^{o}C. Brefeldin A (BFA) was then added to wells to block cytokine transport, allowing cytokines accumulated within the cells. Cells were incubated for a further 15 hours at 37 ^{o}C. Plates were then centrifuged at 1800 rpm for 3 mins. Supernatants were removed and cells were washed using staining buffer. Plates were centrifuged again at 1800 rpm for 3 mins and supernatants were removed. Antibody mixture made up of 1:100 anti-human CD4 FITC, 1:600 anti-human CD8a BV510, 1:50 CD56 BV421, 1:200 CD14 APC-Fire750 and 1:1000 L/D Near Infra-Red (Supplementary Table 8) was prepared in staining buffer and FcR blocking buffer (1:10 dilution). Cells were stained for 20 mins at 4 ^{o}C in the dark and washed cells using staining buffer. For intracellular staining, cells were first fixed using Fix/Perm solution for 20 mins at 4 ^{o}C in the dark. Cells were then washed using Perm/Wash with 2 mins incubation on ice in the dark. Supernatant was removed and cells were ready to be stained. Intracellular Ab mix was prepared: 1:100 anti-human CD3 PerCP, 1:50 IFN-γ PE, 1:100 TNFα APC, 1:160 IL2 PE-Cy7 (Supplementary Table 8) in Perm/Wash and FcR

blocking reagent. Cells were stained for 20 mins at 4 ^{o}C in the dark. Cells were washed in Perm/Wash before resuspended in staining buffer for acquisition on MACS Quant.

2.15 Data Analyses

Chapter 3,4 and 5

Flow data were first analysed in FlowJo v10.8.1. The gating strategy is shown for proliferation assay (Supplementary Figure 1), extracellular staining (Supplementary Figure 2), intracellular staining (Supplementary Figure 3) and immunophenotyping analysis (Supplementary Figure 4).

Graph and data analysis were done in R 4.5.1 and GraphPad Prism 10. Comparisons between two groups were calculated using Wilcoxon rank-sum test, while comparisons between multiple groups were analysed using the Kruskal-Wallis test, followed by post hoc Dunn's test with Bonferroni correction. The difference between two paired groups before and after vaccination timepoints was measured using Wilcoxon signed-rank Test. Correlation analysis was performed using Spearman's test. Univariable logistic regression was performed to assess individual factors and outcome. Multivariable logistic regression analysis was conducted to evaluate the association between demographic characteristics and baseline antibody levels with the likelihood of an increase in antibody response following vaccination.

Additional data analyses in chapter 4

Proteomics data analyses were performed jointly with Clement Twumasi.

Protein biomarker selection should consider biological variability, as some protein expression highly variable between healthy individuals and within individuals over time,

influenced by factors such as age, sex, and genetics, whereas others remain relatively stable which would be more suitable to detect changes due to disease or interventions (Corzett et al., 2010; Dodig-Crnković et al., 2020).

Principal component analysis (PCA) was performed to evaluate overall variance structure. Analysis of serum and plasma was performed using paired samples from the same individuals minimised inter-individual biological confounding factors. Univariable comparisons of paired data were calculated using log2 fold change and p-values from the Wilcoxon rank-sum test were adjusted using the false discovery rate (FDR).

To identify proteins predictive of T-cell response following vaccination, the complete dataset was split into training and test/validation dataset, in order to develop the model and to test the model in different dataset. Using training dataset, feature selection was performed using Least Absolute Shrinkage and Selection Operator (LASSO), which penalised model complexity and reduced collinearity in proteomic data based on their association with the outcome (T-cell response). LASSO-selected proteins were then incorporated into mixed-effects regression model that include age, sex, serostatus to CMV as fixed effects and participant-level random effects. Mixed-effects regression models calculate association between a specific predictor and an outcome while adjusting with other predictors and accounting for data clustering. By including demographic covariates as fixed effects and participant-level random intercepts, the mixed-effects logistic model estimates the association between each protein and T-cell response while adjusting for other confounders. A similar analytic framework has been used to identify proteins predictive of COVID-19 severity (Hwangbo et al., 2022; Su et al., 2023). The model discrimination was assessed using the area under receiver operating characteristic curve. The immune competence score estimator was calculated using the formula below:

$$P(\text{T-responder}) = \frac{e^{\hat{b}_o + \sum_{i=1}^{n} \hat{b}_i X_i}}{1 + e^{\hat{b}_o + \sum_{i=1}^{n} \hat{b}_i X_i}}$$

Xi=specific protein markers of T-cell response (eIF5A, IL1RL2, COL9A1, MMP-1, SKAP2, Age, Sex and IgG CMV)

3 Antibody and T-cell responses to SARS-CoV-2 and seasonal human coronaviruses in vaccinated UK healthcare workers who self-reported with no known infection

3.1 Introduction

Healthcare workers (HCWs) faced a high risk of SARS-CoV-2 exposure during the pandemic due to their frontline role in patient care. HCWs were among the first group of population to receive COVID-19 vaccines, and they were also a population that was continuously screened for SARS-CoV-2 infection due to the risk of their occupation and the need to protect patients and colleagues from SARS-CoV-2 transmission. Despite their exposure risk, a subset of HCWs reported never having been infected with SARS-CoV-2 (Swadling et al., 2022), prompting interests regarding the factors associated with resistance to infection.

Vaccination with spike-based COVID-19 vaccines, including mRNA vaccines and viral vector vaccines, induces immune response to spike (S) protein. Antibodies to S have neutralising capability (Piccoli et al., 2020), inhibiting SARS-CoV-2 entry into host cells *in vitro* (Hoffmann et al., 2020). Selective pressure from the host immune system has caused S region to mutate (McCallum et al., 2021) and to escape antibody recognition. T-cells, on the other hand, can recognise a broader range of epitopes across the spike protein and can therefore provide cross-protection against variants of concern (Geers et al., 2021; Moore et al., 2023; Skelly et al., 2021; A. Tarke et al., 2021).

In contrast, people infected with SARS-CoV-2 typically developed IgG antibody response not only to S but also to other viral proteins, such as nucleocapsid, NSP1, NSP7, NSP8, RNA-dependent RNA polymerase (RdRp) (NSP12), ORF3b, ORF9b (Li et al., 2021).

Following infection, T-cells were also generated to both spike and non-spike proteins. $CD4^+$ T-cell responses are predominantly directed against the structural proteins S, M and N, due to the abundance of these proteins, while $CD8^+$ T-cells are generated to S and N, but less frequently to M (Ogbe et al., 2021; Alison Tarke et al., 2021).

Intramuscular COVID-19 vaccines can elicit detectable mucosal immune responses (Sheikh-Mohamed et al. 2022; Stolovich-Rain et al. 2023), which are important for defence against SARS-CoV-2. Higher IgA levels have been associated with protection (Sheikh-Mohamed et al., 2022). However, compared with natural infection, intramuscular vaccination induces limited mucosal immune responses (Tang et al., 2022). The observed compartmentalisation between nasal IgA and plasma antibody responses following intramuscular vaccination, likely reflects local antigen exposure rather than systemic vaccine-induced immunity (Liew et al., 2023).

SARS-CoV-2 share regions of sequence homology with human seasonal coronaviruses (HCoVs), which can generate cross-reactive immune responses (Anderson et al., 2021). The functional implications of this cross-reactivity, especially antibodies, remain uncertain (Murray et al., 2023). IgG to HCoVs has been reported to be higher in asymptomatic patients compared with symptomatic ones (Ortega et al., 2021), whereas another study demonstrated that pre-existing antibodies to seasonal coronaviruses correlated with increased susceptibility to SARS-CoV-2 infection (Wratil et al., 2021). In contrast, exposure to endemic human coronaviruses (HCoVs) generated T-cells targeting conserved viral replication and transcription complex (RTC), such as NSP7, NSP8 and NSP12, which have been reported to provide immunity to SARS-CoV-2 (Swadling et al., 2022).

Based on these findings, I hypothesised that vaccinated healthcare workers (HCWs) who self-reported with no confirmed SARS-CoV-2 infection may have robust spike-specific antibody and T-cell responses following their vaccination, as well as T-cell reactivity to conserved replication-transcription complex (RTC) antigens, while a subset may have experienced unrecorded viral exposure, as reflected by non-spike-directed humoral and T-cell immunity and mucosal immune responses. The aim of this chapter was therefore to characterise systemic and mucosal humoral and cellular immune responses in vaccinated HCWs without a reported history of SARS-CoV-2 infection, and to compare these profiles with early pandemic samples and individuals with confirmed breakthrough infection.

3.2 Results

3.2.1 Study population

Samples were collected from HCWs across mainland UK (Table 3-1). Participants were aged 21-70 years, and more than 78% were female. There were three groups of samples (Figure 3-1): 1) Archived samples collected between April 2020 and June 2020 from 23 unexposed and unvaccinated participants (UE). UE participants were tested negative by ELISA for anti-SARS-CoV-2 S glycoprotein IgG/A/M antibodies (Faustini et al., 2021) and the blood was drawn during the first lockdown when the proportion of the population who had been infected was low; 2) HCWs vaccinated with SARS-CoV-2 vaccines who self-reported with no known SARS-CoV-2 infection (No Known Infection (NKI)) (n=75). Samples were collected between May 2022 and April 2023 and sampling timepoints are heterogeneous relative to vaccination; 3) Vaccinated individuals in the PITCH study in Oxford who had polymerase chain reaction (PCR) or lateral flow test (LFT) confirmed breakthrough infection 15-30 days earlier (Documented Infection (DI)) (n=23). Samples were collected between January 2022 and December 2023.

Most vaccinated participants in both the NKI and DI groups were sampled more than six months after their third or fourth vaccination (44% [33/75] NKI; 52% [12/23] DI), with smaller proportions sampled within three to six months (37% [28/75] NKI; 30% [7/23] DI) or less than three months post-vaccination (12% [9/75] NKI; 17% [4/23] DI).

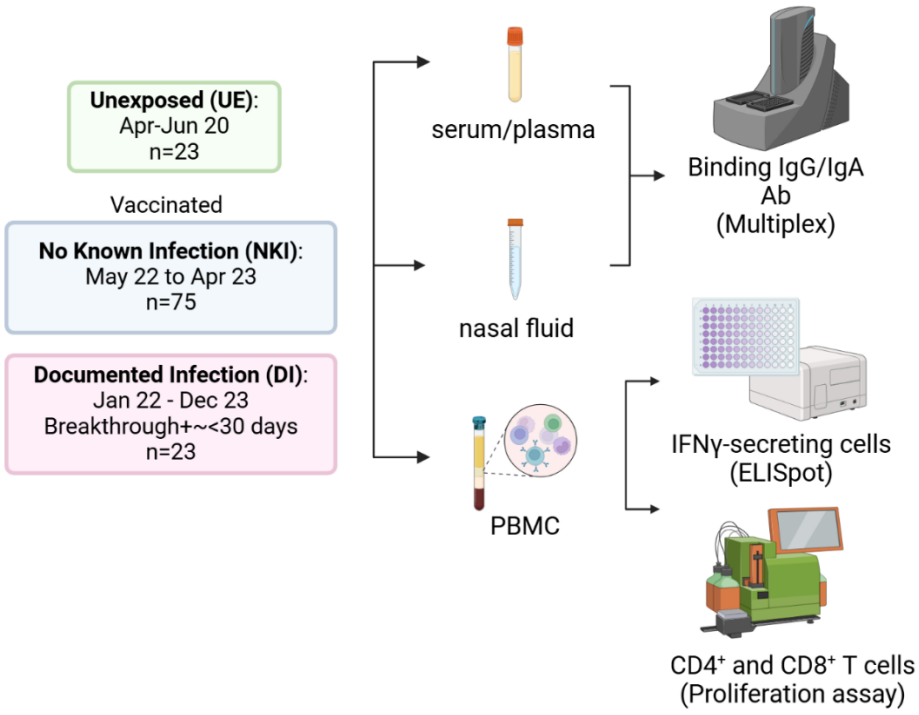

Figure 3-1 Study overview

Study overview. Blood samples and nasal fluid were collected from UK healthcare worker (HCWs). Unvaccinated and unexposed group (UE) samples were collected between April-June 2020. Samples from vaccinated individuals with no known infection (NKI) or with documented infection (DI) were collected during the pandemic. Antibody and T-cell responses to SARS-CoV-2 and seasonal human coronaviruses (HCoVs) were measured and compared between the study groups. *Created with Biorender.com.*

Table 3-1 Demographic characteristics of study population

	Unvaccinated	**Vaccinated**		
	Unexposed (UE) (n=23)	**Detected Infection (DI) (n=23)**	**No Known Infection (NKI) (n=75)**	**p-value***
Age, year (range, mean ± SD)	21-69 years, 40.3±13.3	21-63 years, 40.7±12.1	21-70 years, 47.9±12.3	0.012
Sex, n (%)				≥0.05
Male	3 (13%)	5 (21.7%)	15 (21.7%)	
Female	20 (87%)	18 (78.3%)	54 (78.2%)	

*Kruskal-Wallis, Fisher's exact test

3.2.2 Vaccinated individuals had SARS-CoV-2 spike-specific systemic IgG antibodies

Given that the NKI group had received spike-based COVID-19 vaccines, most participants, 97.1% (67/69), in the NKI group had IgG antibodies to SARS-CoV-2 S (Figure 3-2, Table 3-2). The level of antibody response to spike proteins was not significantly different between the NKI group (median: 290,836 AU/mL) and the DI group (median: 576,996 AU/mL) ($p \geq 0.05$) (Figure 3-2). A small proportion of the UE group, 2/19 (10.5%), also had antibody to SARS-CoV-2 spike (Table 3-2). IgG to RBD levels did not differ significantly between the NKI (median: 159,115 AU/mL) and the DI (median: 411,835 AU/mL) groups, whereas the DI group (median: 7,553 AU/mL) had higher IgG to NTD than the NKI group (median: 3,697 AU/mL) ($p=0.02$).

3.2.3 One-third of the NKI group had SARS-CoV-2 nucleocapsid-specific systemic IgG antibodies

IgG to N levels in the blood were measured as a serological marker of prior SARS-CoV-2 infection. Twenty out of 67 (29.9%) individuals in the NKI group and 1/19 (5.3%) in the UE group had IgG to SARS-CoV-2 N (Figure 3-2, Table 3-2). Compared with the DI group (median: 62,010 AU/mL), the NKI group (median: 638 AU/mL) had significantly lower IgG to N ($p<0.001$), but higher than the UE group (median: 129.1 AU/mL).

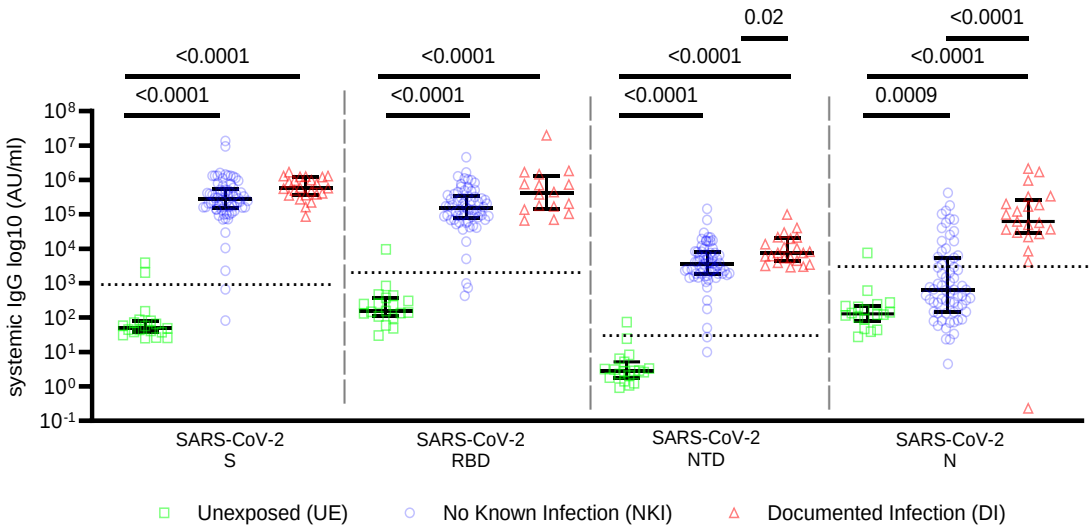

Figure 3-2 Systemic IgG responses against SARS-CoV-2

Binding IgG responses in plasma or serum of UE (n=21-23; green squares), NKI (n=67-69; blue dots) and DI groups (n=19; red triangles) were measured using the MesoScale Discovery (MSD) platform. Antibodies were measured against SARS-CoV-2 spike (S), SARS-CoV-2 receptor binding domain (RBD), SARS-CoV-2 N-terminal domain (NTD), SARS-CoV-2 nucleocapsid (N). Each data point represents an individual sample. Error bars represent the median with interquartile range. Comparison between groups were performed using the Kruskal-Wallis with a post-hoc analysis. Dotted lines indicate the seropositivity cut-offs. No seropositivity cut-off was available for IgG to NL63 S, HKU1 S, OC43 S or 229E S.

Table 3-2 Number of participants positive for systemic SARS-CoV-2-specific IgGs

Systemic IgG	Unexposed (UE)	Documented Infection (DI)	No Known Infection (NKI)	p-value*
CoV-2 S	2/19 (10.5%)	23/23 (100%)	67/69 (97.1%)	<0.001
CoV-2 RBD	1/19 (5.3%)	16/16 (100%)	61/64 (95.3%)	<0.001
CoV-2 NTD	1/19 (5.3%)	22/22 (100%)	65/67 (97.0%)	<0.001
CoV-2 N	1/19 (5.3%)	21/21 (100%)	20/67 (29.9%)	<0.001

*Fisher's exact test

3.2.4 Nasal IgG levels to SARS-CoV-2 S was not significantly different between the NKI and DI groups

I next analysed binding IgG and IgA antibodies in nasal samples from NKI (n=53) and DI (n=12) groups. Some datapoints were excluded because the results fell outside the assay's

detection range. Nasal samples from the UE group were unavailable, but the positive cut-off set by the assay was measured using pre-pandemic nasal samples (n=4-5).

Almost all participants in the NKI group, >95%, had nasal IgG to SARS-CoV-2 S, RBD and NTD (Figure 3-3a, Table 3-3). Nasal IgG to S did not differ significantly between NKI (median: 821 AU/mL) and DI groups (median: 968 AU/mL) ($p \geq 0.05$) (Figure 3-3a). Some of the NKI group had anti-N IgG, although the levels were lower (median: 2.0 AU/mL) compared with the DI group (median: 92.1 AU/mL) ($p < 0.05$). The proportion of NKI participants who were positive for SARS-CoV-2 N was 31.4% (16/5) (Table 3-3). In contrast, 9/12 (75%) DI participants were positive for nasal IgG to N, consistent with a previous report showing 64.52%-78.95%, of individuals developed nasal IgG to N by 28 days after mild infection, while the majority (>90%) generated nasal IgG to S (Fröberg et al., 2021).

Most participants in the NKI group had nasal IgA to SARS-CoV-2 S, RBD and NTD (Figure 3-3b, Table 3-3). Nasal IgA responses to spike were significantly lower in the NKI group (median: 15.53 AU/mL) than the DI group (median: 48.52 AU/mL) ($p < 0.05$) (Figure 3-3b). A similar pattern was observed for nasal IgA to RBD, NTD, and N. Nasal IgA to N was detected in 33/45 (73.3%) individuals in the NKI group, compared with 11/12 (91.7%) individuals in the DI group (Table 3-3). While mucosal IgA responses were detected in the NKI group, higher nasal IgA levels against both spike and non-spike were observed in individuals with documented infection.

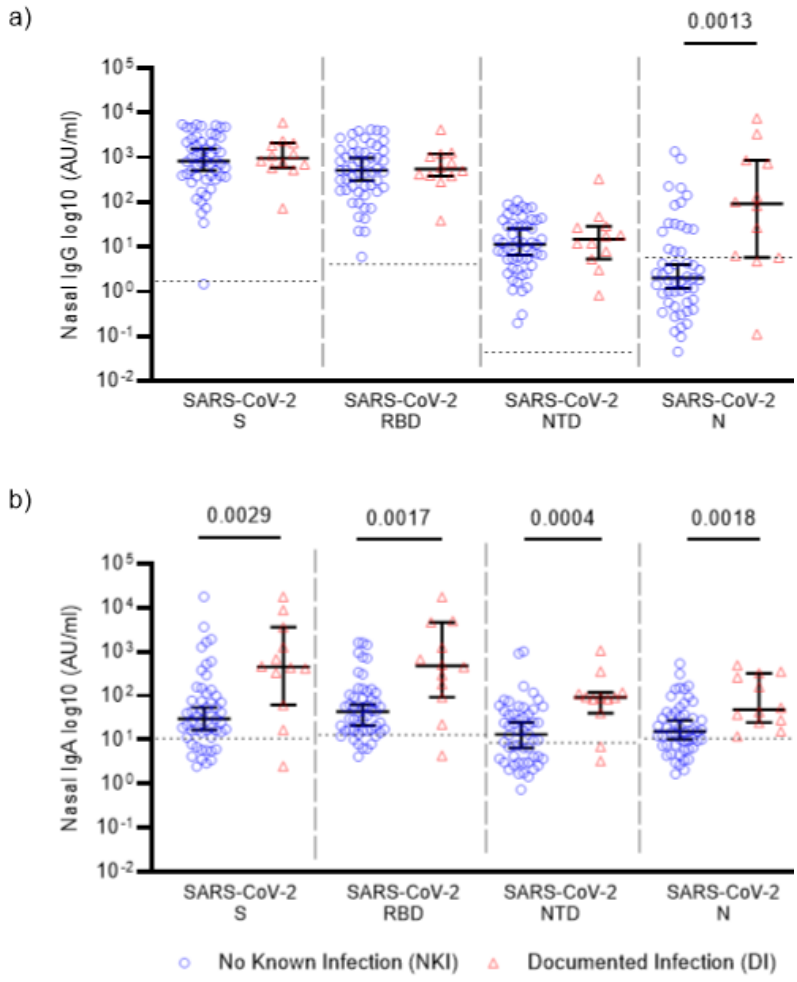

Figure 3-3 Nasal IgG and IgA responses against SARS-CoV-2

a) IgG and b) IgA responses in nasal lining fluid from the NKI group (n=51-53; blue dots) and the DI group (n=12; red triangles) were measured using the MSD platform. Antibody responses were measured against SARS-CoV-2 spike (S), receptor binding domain (RBD), N-terminal domain (NTD), nucleocapsid (N). Each data point represents an individual sample. Error bars represent the median with interquartile range. Comparisons between groups were performed using the Wilcoxon rank-sum test. Dotted lines indicate the positive cut-off for each antigen-specific antibody.

Table 3-3 Number of participants positive for nasal SARS-CoV-2-specific antibodies

	No Known Infection (NKI)	Documented Infection (DI)	p-value*
Nasal IgG			
CoV-2 S	52/53 (98.1%)	12/12 (100%)	≥0.05
CoV-2 RBD	53/53 (100%)	12/12 (100%)	≥0.05
CoV-2 NTD	51/53 (96.2%)	12/12 (100%)	≥0.05
CoV-2 N	16/51 (31.4%)	9/12 (75%)	<0.01
Nasal IgA			
CoV-2 S	42/53 (79.2%)	11/12 (91.7%)	≥0.05
CoV-2 RBD	40/44 (90.1%)	11/12 (91.7%)	≥0.05
CoV-2 NTD	31/45 (68.9%)	10/12 (83.3%)	≥0.05
CoV-2 N	33/45 (73.3%)	11/12 (91.7%)	≥0.05

*Fisher's exact test

3.2.5 Correlation between systemic and nasal antibody responses in the NKI group

Correlations between systemic and nasal antibody responses were assessed in the NKI group to evaluate whether systemic antibodies were reflected in the nasal compartment or whether nasal responses were indicative of asymptomatic infection. Systemic SARS-CoV-2 S-specific IgG (sIgG S) showed a moderate correlation with nasal S-specific IgG (nIgG S) ($r = 0.53$, $p = 0.001$) and a weaker correlation with nasal S-specific IgA (nIgA S) ($r = 0.38$, $p = 0.01$) (Figure 3-4). sIgG S was also correlated with systemic N-specific IgG (sIgG N) ($r = 0.59$, $p = 0.001$), but showed only weak correlations with nasal N-specific IgG (nIgG N) or IgA (nIgA N). sIgG N, a serological indicator consistent with prior infection, moderately correlated with nIgG N ($r=0.49$, $p=0.001$), but not with nIgA N. Taken together, systemic IgG responses showed stronger correlations with nasal IgG than with nasal IgA, indicating a closer alignment between systemic and nasal IgG responses, whereas nasal IgA responses were more weakly associated with systemic immunity.

Within the nasal compartment, nIgG S moderately correlated with nIgA S (r=0.61, p=0.001) and nIgG N correlated with nIgA N (r=0.58, p=0.01), indicating coordinated local antibody response across antibody isotypes. Cross-antigen associations were also observed: nIgG S moderately correlated with nIgG N (r=0.57, p=0.001) and weakly with nIgA N (r=0.22, p<0.05), while nIgA S moderately correlated with nIgA N (r=0.51, p<0.001).

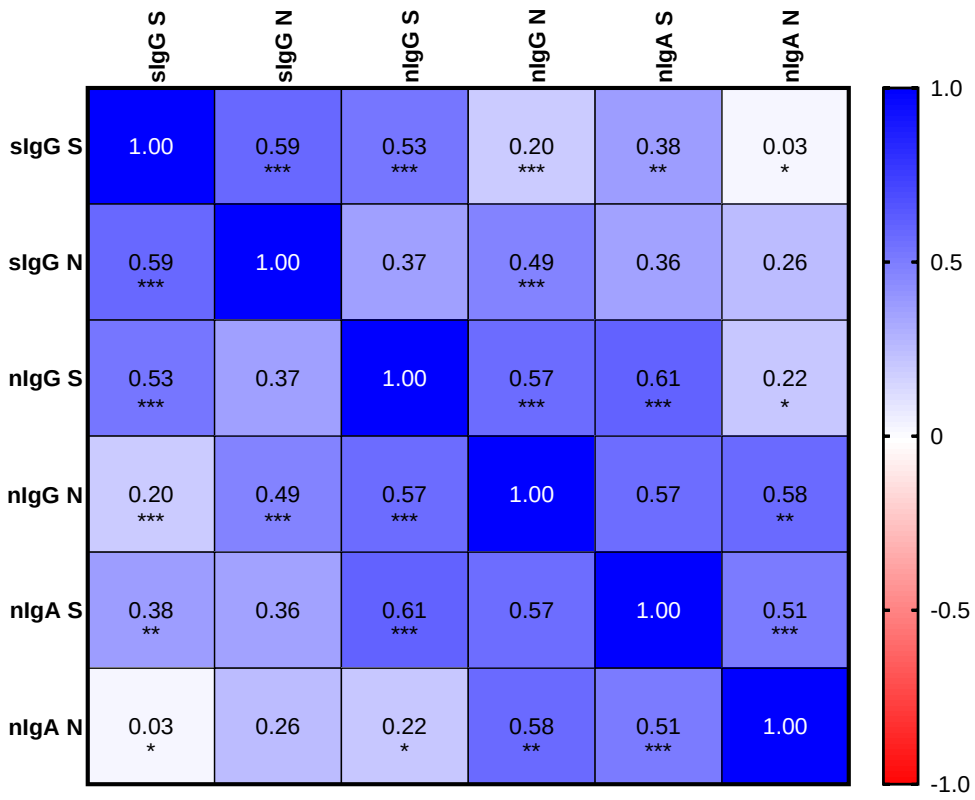

Figure 3-4 Heatmap showing correlations between systemic and nasal antibodies in the NKI group

Correlations between systemic and nasal antibodies responses in the no known infection (NKI) group (n≈53) were analysed using Spearman's test. Number within each square represents correlation coefficient. Blue indicates the strongest positive correlation and red indicates the strongest negative correlation. P-value was indicated as: *=<0.05, **=<0.01, ***=<0.001.

3.2.6 Systemic and nasal antibody responses to endemic coronavirus spike proteins in the NKI group

Cross-reactive immune responses to SARS-CoV-2 may influence susceptibility to infection, either positively or negatively. I measured IgG in the blood to SARS-CoV-1 and to four HCoVs and compared the responses across groups (Figure 3-5). Cut-off values were only available for SARS-CoV-1-specific antibodies, as information on exposure to seasonal coronaviruses was not available for the pre-pandemic samples.

Levels of SARS-CoV-1 S- and HCoV S-specific IgGs in the blood were not significantly different between the NKI and DI groups. Based on the very low probability of SARS-CoV-1 exposure in this cohort, SARS-CoV-1-specific IgG was antibody responses observed in the NKI and DI groups are most likely vaccine-induced, given the high homology (Geanes et al., 2022), rather than reflective of pre-existing immunity. In contrast, the UE group had no IgG to SARS-CoV-1 and lower levels of antibodies to HCoVs than the NKI and DI groups.

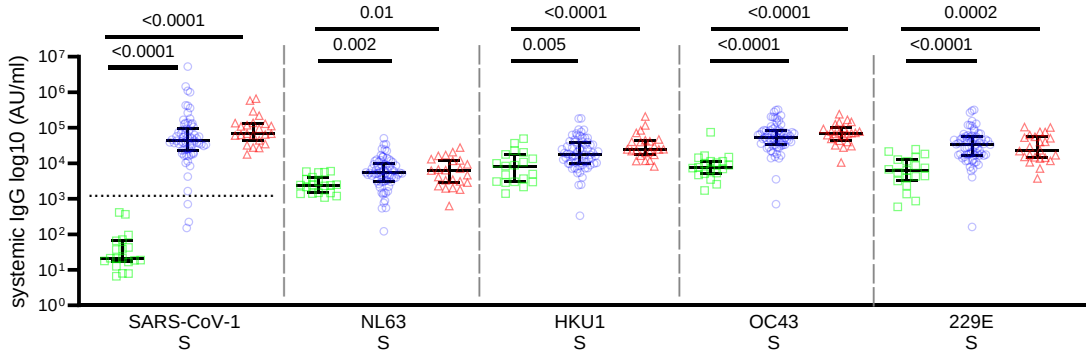

Figure 3-5 Systemic IgG responses against SARS-CoV-1 and HCoVs

Binding IgG responses in plasma or serum of UE (n=21-23; green squares), NKI (n=67-69; blue dots) and DI groups (n=19; red triangles) were analysed using MesoScale Discovery (MSD). Antibodies were measured against SARS-CoV-1 S and HCoVs. Each dot represents binding IgG antibody data from a single sample. Error bars represent the median with interquartile range. Comparison between groups were made using the Kruskal-Wallis with post-hoc analysis. The dotted line indicates the seropositivity cut-off. No seropositivity cut-off is available for IgG to NL63 S, HKU1 S, OC43 S or 229E S.

In the nasal compartment, levels of SARS-CoV-1 S-specific IgG did not differ between the NKI and DI groups (Figure 3-6a), whereas nasal IgA levels were significantly lower in the NKI group (Figure 3-6b), a similar pattern that was observed for SARS-CoV-2 S-specific nasal IgG (Figure 3-3a). Levels of nasal IgG and IgA to HCoVs did not differ significantly between the NKI and DI groups (Figure 3-6a,b).

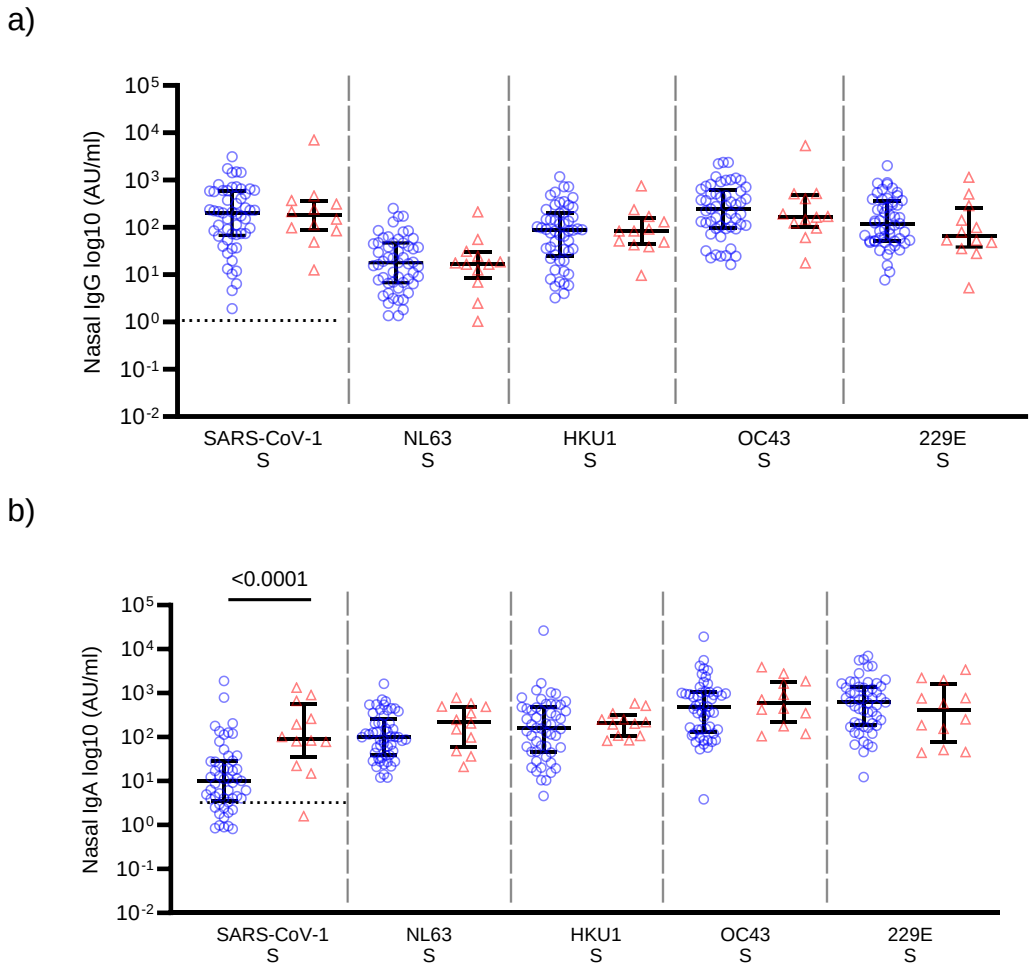

Figure 3-6 Nasal binding IgG and IgA responses against SARS-CoV-1 and HCoVs

a) IgG and b) IgA responses in nasal lining fluid from the NKI group (n=51-53; blue dots) and the DI group (n=12; red triangles) were analysed using the MSD platform. Antibody responses were measured against SARS-CoV-1 S, NL63 S, HKU1 S, OC43 S and 229E S. Each data point represents an individual sample. Error bars indicate the median with interquartile range. Comparisons between groups were performed using the Wilcoxon rank-sum test. Dotted lines indicate the positive cut-off for each antigen-specific antibody. No cut-off values were available for antibodies against NL63 S, HKU1 S, OC43 S and 229E S.

To further examine the relationship between antibody responses to SARS-CoV-2 and seasonal human coronaviruses (HCoVs), I performed Spearman's correlation analysis. Moderate correlations ($r = 0.3\text{-}0.4$, $p < 0.05$) were observed between IgG to SARS-CoV-2 S and IgG to HCoV S antigens in the NKI group. In the DI group, IgG to SARS-CoV-2 S correlated strongly with IgG to other betacoronaviruses, including HKU1 ($r = 0.68$, $p < 0.05$) and OC43 S ($r = 0.67$, $p < 0.05$) (Table 3-4a). No significant correlations were observed in the UE group.

A similar pattern was found in the nasal compartment. The NKI group showed moderate correlations between nIgG to SARS-CoV-2 S and nIgG to HCoV spike antigens, whereas the DI group demonstrated a strong correlation ($r = 0.76$, $p < 0.05$) between nIgG to SARS-CoV-2 S and nIgG to OC43 S. (Table 3-4b). No meaningful correlations were observed between nIgA to SARS-CoV-2 and nIgA to HCoVs (Table 3-4c).

Table 3-4 Correlation of SARS-CoV-2 S- and HCoV- specific antibodies

a)

sIgG to SARS-CoV-2 S	229E S	NL63 S	HKU1 S	OC43 S
UE				
NKI	0.34	0.43	0.46	0.38
DI			0.68	0.67

b)

nIgG to SARS-CoV-2 S	229E S	NL63 S	HKU1 S	OC43 S
NKI	0.44	0.5	0.68	0.66
DI				0.76

c)

nIgA to SARS-CoV-2 S	229E S	NL63 S	HKU1 S	OC43 S
NKI				
DI				

3.2.7 IFN-γ ELISPOT responses to SARS-CoV-2 S did not differ significantly between the NKI and DI groups

T-cell responses to S and non-S peptides were measured using IFN-γ ELISpot assays. ELISpot results from 29/88 samples had to be excluded because of suboptimal sample quality due to a freezer failure at the Peter Medawar Building. The excluded samples did not pass our quality control checks required before data analysis of cellular responses, either showing high background in negative control wells or low cell counts in positive control wells (Section 2.7).

S-specific IFN-γ secreting cells did not differ significantly between the NKI (median: 98 SFU/10^6 PBMCs) and DI (median: 60 SFU/10^6 PBMCs) groups ($p \geq 0.05$) (Figure 3-7). Most participants in the NKI (80.6%) and DI (76.5%) groups had T-cell responses to S (Figure 3-7). The NKI did not showed T-cell responses to M and N (median: 13 SFU/10^6 PBMCs) that was higher than the UE group (median: 4 SFU/10^6 PBMCs), unlike the DI group (median: 30 SFU/10^6 PBMCs). There were no differences in IFN-γ secreting cells against non-structural and accessory proteins among groups (Figure 3-7). Sixteen out of 31 (51.6%) of the NKI group had T-cell responses to non-spike proteins by ELISpot assay (Figure 3-7).

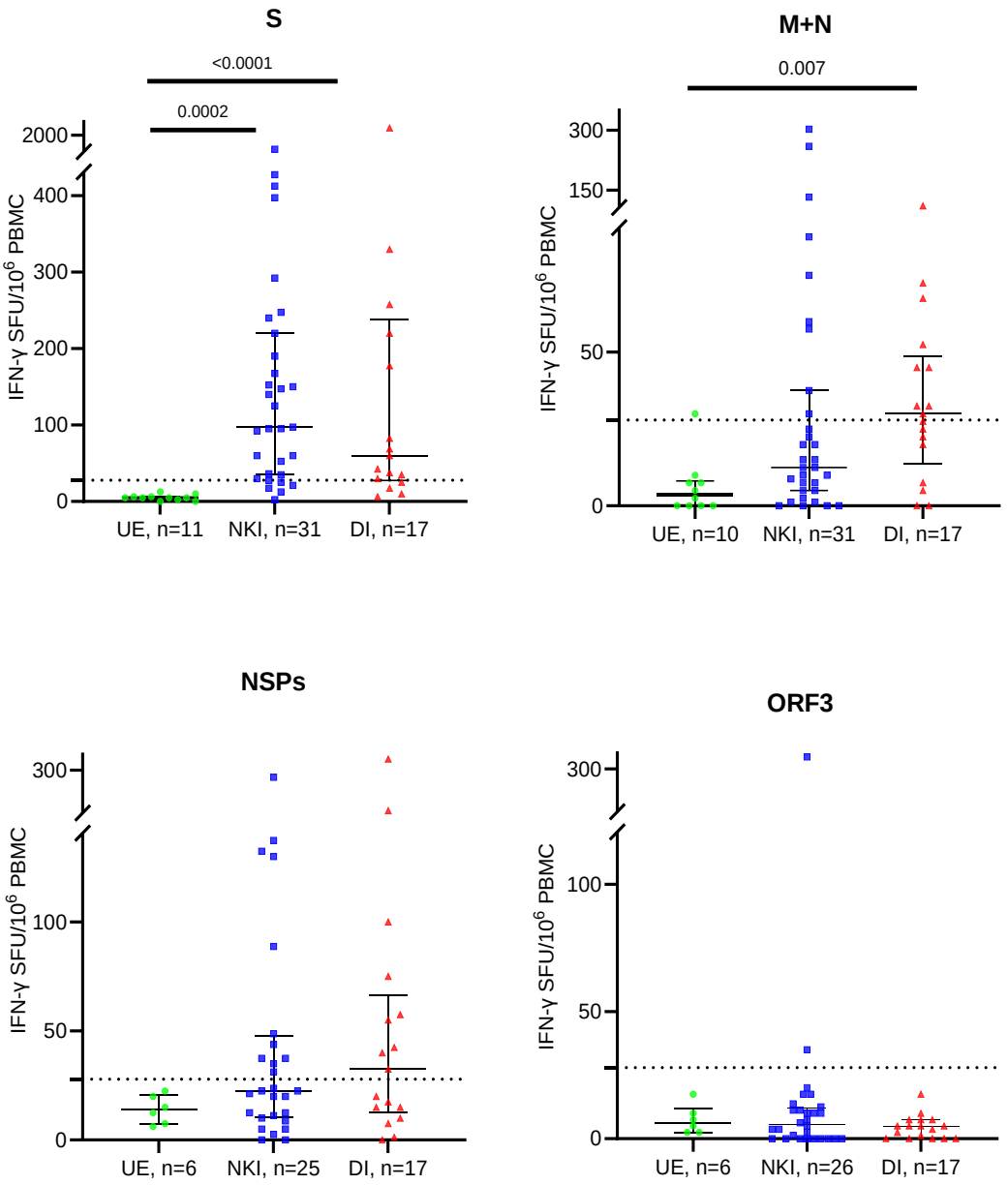

Figure 3-7 IFN-γ ELISpot responses to SARS-CoV-2 spike and non-spike proteins

PBMCs (unexposed (UE) n=11; No Known Infection (NKI) n=31; Documented Infection (DI) n=17) were stimulated for 16-18 hours with overlapping SARS-CoV-2 peptides, including spike (S), membrane and nucleocapsid proteins (M+N), non-structural proteins (NSP3, 7-11, 12, 13), and open reading frame (ORF)3. IFN-γ spot forming unit (SFU) per 1 million cells are shown. The cut-off was set at the mean background + 2SD (dashed line). Comparisons between groups were performed using Kruskal Wallis with post-hoc Dunn's test.

Table 3-5 IFN-γ ELISpot responses to SARS-CoV-2 spike and non-spike proteins

Type of peptides recognised	Unexposed (UE) (n=1/11)	No Known Infection (NKI) (n=26/31)	Documented Infection (DI) (n=15/17)	p-value*
S	0/11 (0%)	25/31 (80.6%)	13/17 (76.5%)	<0.05
non-spike (M+N, NSPs or ORF3)	1/10 (10%)	16/31 (51.6%)	13/17 (76.5%)	<0.05
M+N	1/10 (10%)	9/31 (29%)	9/17 (52.9%)	≥0.05
NSPs	0/6 (0%)	12/25 (48%)	9/17 (52.9%)	≥0.05
ORF3	0/6 (0%)	2/26 (7.7%)	0/17 (0%)	≥0.05

*Fisher's exact test

3.2.8 CD4+ and CD8+ T-cell proliferative responses to S and non-spike proteins were detected in the NKI group

To characterise T-cell responses, the proliferative frequency of CD4+ and CD8+ T-cells was measured using a CellTrace Violet (CTV) dye-based proliferation assay. The NKI group had higher CD4+ T-cell responses to S1, S2, and N compared with the UE group (p<0.05) (Figure 3-8). CD8+ T-cells to S1 (median: 47.6%) were also higher in the NKI group than the UE group (median: 20.8%) (p<0.05) (Table 3-6). Twenty of 51 (39%) individuals in the NKI group had a $CD4^+$ T-cell response to S (Table 3-6) and 22/51 (43%) also had a $CD8^+$ T-cell response to S (Figure 3-9, Table 3-6). Six out of 15 individuals, 40%, in the DI group had a proliferative response to any spike or non-spike proteins tested, despite recent infection (Table 3-6). The DI group had higher $CD4^+$ T-cell responses to N and $CD8^+$ T-cell responses to S1 compared with the UE group (p<0.05) (Figure 3-8, Figure 3-9).

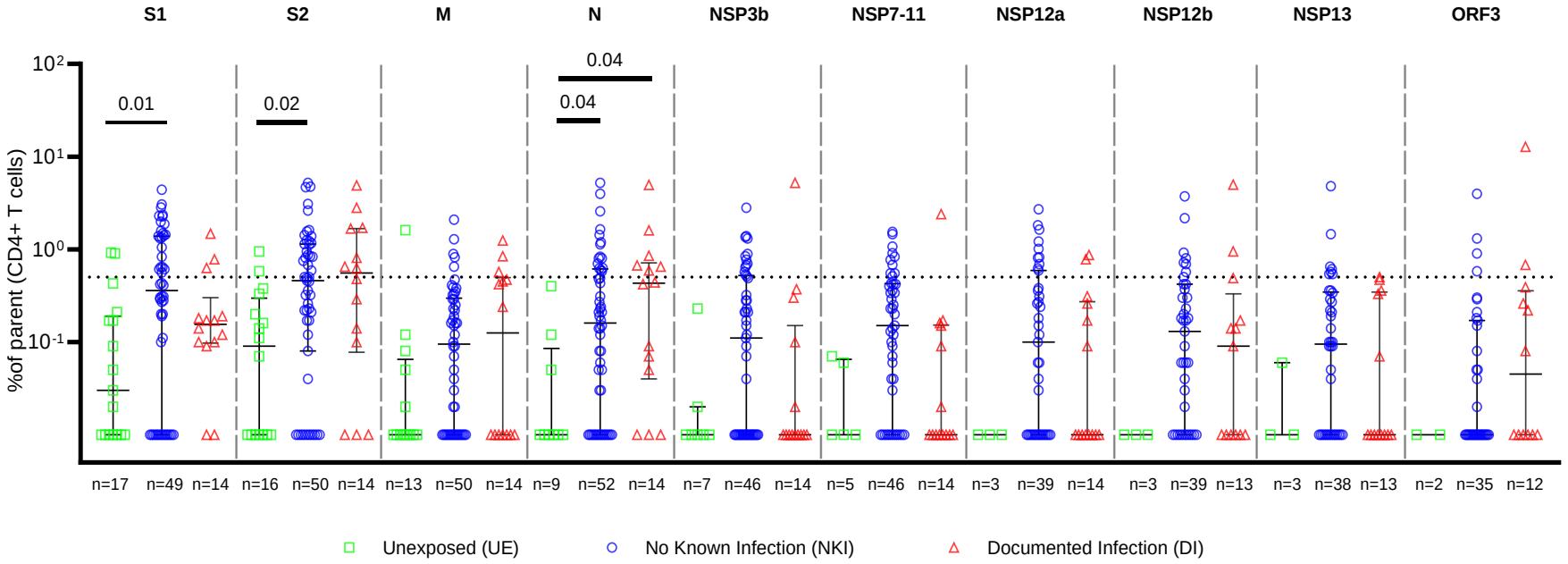

Figure 3-8 CD4⁺ T-cell proliferative responses to SARS-CoV-2 proteins

PBMC were stimulated for 7 days with overlapped SARS-CoV-2 peptides, including Spike (S)1, S2, Membrane (M), and nucleocapsid proteins (N), non-structural proteins (NSP) 3b, NSP7-11, NSP12a, NSP12b, NSP13, and open reading frame (ORF)3. Each dot is the proliferation percentage of CD4+ T-cell to parent after subtracted to responses detected in the background well. Median with 95% CI is also presented in the graph. The cut-off was set at 0.5% (dashed line). Comparison between groups was measured using Kruskal-Wallis with post-hoc Dunn's test.

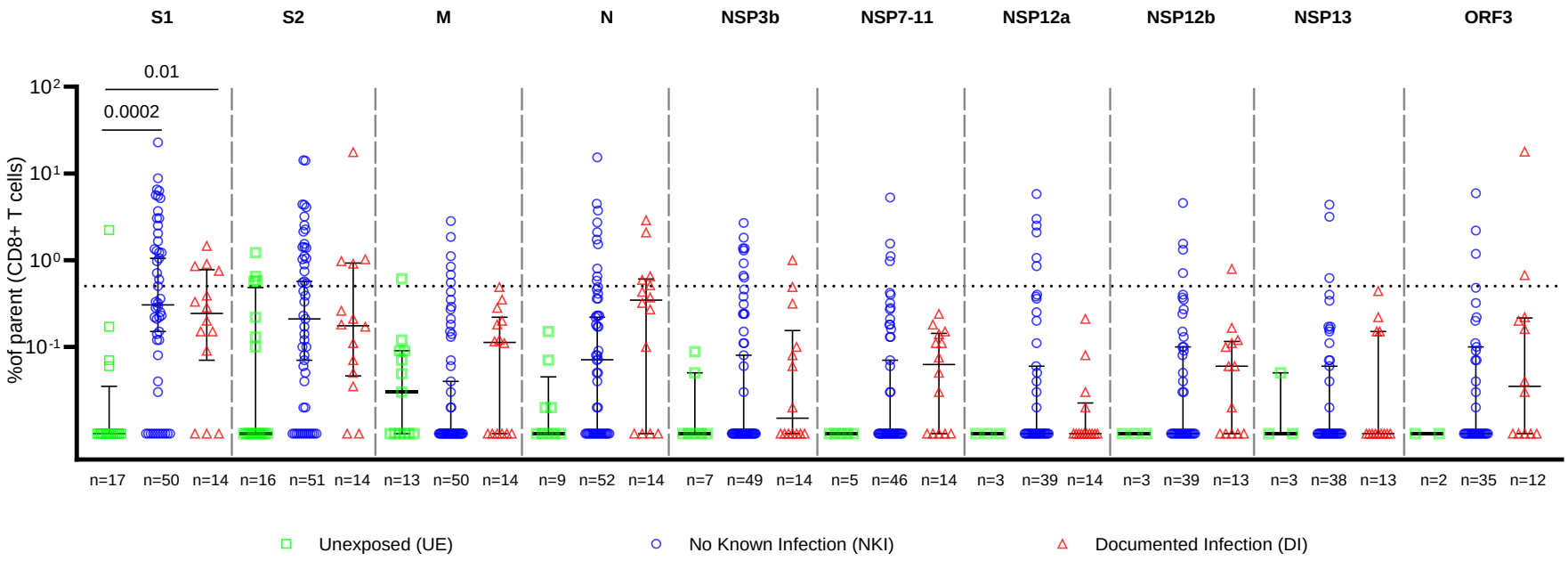

Figure 3-9 CD8⁺ T-cell proliferative responses to SARS-CoV-2 proteins

PBMC were stimulated for 7 days with overlapped SARS-CoV-2 peptides, including Spike (S)1, S2, Membrane (M), and nucleocapsid proteins (N), non-structural proteins (NSP) 3b, NSP7-11, NSP12a, NSP12b, NSP13, and open reading frame (ORF)3. Each dot is the proliferation percentage of CD8+ T-cell to parent after subtracted to responses detected in the background well. Median with 95% CI is also presented in the graph. The cut-off was set at 0.5% (dashed line). Comparison between groups was measured using Kruskal-Wallis with post-hoc Dunn's test.

Table 3-6 CD4+ T-cell and CD8+ T-cell proliferative responses to SARS-CoV-2 peptides

	Unexposed (UE) (n=1/17)	No Known Infection (NKI) (n=31/51)	Documented Infection (DI) (n=6/15)	P value*
Type of peptides recognised by CD4+ T-cells				
S (S1 or S2)	0/17 (0%)	20/51 (39%)	4/15 (27%)	<0.01
non-S (M or N or NSPs or ORF3)	1/17 (5.9%)	18/51 (35%)	4/15 (27%)	≥0.05
	Unexposed (UE) (n=2/17)	**No Known Infection (NKI) (n=28/51)**	**Documented Infection (DI) (n=6/15)**	
Type of peptides recognised by CD8+ T-cells				
S (S1 or S2)	2/17 (12%)	22/51 (43%)	4/15 (27%)	≥0.05
non-S (M or N or NSPs or ORF3)	0/17 (0%)	14/51 (27%)	3/15 (20%)	<0.05

*Fisher's exact test

3.2.9 Comparison of immune indicators of SARS-CoV-2 infection in the NKI and DI groups

In total there were 75 participants in the NKI group (Figure 3-10), 37 participants had complete antibody (blood and nasal antibodies) and T-cell (ELISpot and proliferation assay) data available (Table 3-7). Of these, 35 individuals had detectable non-spike-specific immune responses in at least one assay. In contrast, two individuals showed no detectable non-spike-specific antibody or T-cell responses across any assay, representing the only participants in this cohort with no immunological evidence suggestive of prior infection (Table 3-7), as observed in the UE group (Table 3-9). These two individuals were additionally analysed longitudinally to characterise spike- and non-spike-specific immune responses across all assays (Appendices 10.3).

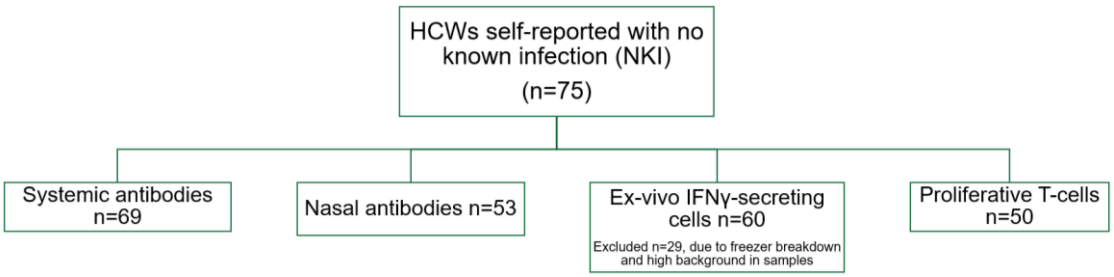

Figure 3-10 Number of NKI samples analysed across assays

The number of samples from the NKI group included in each assay is shown. Antibody and T-cell responses were measured where samples were available, which samples sizes were varied across assays.

In the DI group, all participants exhibited at least two positive non-spike immune response markers. 4/6 (66.67%) DI participants had nucleocapsid-specific antibodies in both blood and nasal fluid, as well as T-cells to non-S (Table 3-8). The other two participants were positive for nucleocapsid-specific systemic IgG and nasal IgA, but negative for nasal IgG, with (n=1) or without T-cell responses to non-spike (n=1).

In the NKI group, 7/37 (18.9%) NKI participants exhibited immune profiles comparable to those observed in the DI group, which may indicate recent infection. In contrast, it remains unclear if other participants in the NKI group with non-spike-specific response detected in one of the assays or two assays, compromising of non-spike-specific antibody response detected only in blood or in nasal fluid, with or without non-spike T cell responses had prior unrecognised exposure to SARS-CoV-2 or cross-reactive responses to human seasonal coronaviruses.

Table 3-7 Numbers of HCWs with or without immune indicators of exposure to SARS-CoV-2

Systemic IgG to N	-	-	-	-	+	-	-	+	+	+	+	+
Nasal IgG to N	-	-	-	+	-	-	+	-	-	+	-	+
Nasal IgA to N	-	-	+	-	-	+	-	-	+	+	+	+
T-cells to non-S	-	+	-	-	-	+	+	+	-	-	+	+
Total (n=37)	2	7	7	1	1	7	1	1	3	3	0	4
Proportion	5.1%	18.0%	18.0%	2.6%	2.6%	18.0%	2.6%	2.6%	7.7%	7.7%	0.0%	10.3%

Legend: N = nucleocapsid; S = spike; + = positive response above positive cut-offs; - = negative response below cut-offs

Table 3-8 Numbers of HCWs in the DI group with or without immune indicators of exposure to SARS-CoV-2

Systemic IgG to N	-	-	-	-	+	-	-	+	+	+	+	+
Nasal IgG to N	-	-	-	+	-	-	+	-	-	+	-	+
Nasal IgA to N	-	-	+	-	-	+	-	-	+	+	+	+
T-cells to non-S	-	+	-	-	-	+	+	+	-	-	+	+
Total (n=6)	0	0	0	0	0	0	0	0	1	0	1	4
Proportion	0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	16.7%	0.0%	16.7%	66.7%

Legend: N = nucleocapsid; S = spike; + = positive response above positive cut-offs; - = negative response below cut-offs

Table 3-9 Numbers of HCWs in the UE group with or without immune indicators of exposure to SARS-CoV-2

Systemic IgG to N	-	-	+	+
Nasal IgG to N	NA	NA	NA	NA
Nasal IgA to N	NA	NA	NA	NA
T-cells to non-S	-	+	-	+
Total (n=4)	4	0	0	0
Proportion	100%	0%	0%	0%

Legend: N = nucleocapsid; S = spike; + = positive response above positive cut-offs; - = negative response below cut-offs

3.2.10 Immune responses between HCWs with patient facing versus non-patient facing roles did not differ significantly

A study of HCWs in Birmingham reported that HCWs working in intensive care units were at lower risk of SARS-CoV-2 infection, which may be due to enhanced personal protective equipment (PPE), training and adherence to training compared with other hospital personnel, such as general medicine staff or housekeepers (Shields et al., 2020). Comparison between HCWs in patient-facing and non-patient facing roles in our cohort (Supplementary Figure 5, Supplementary Table 9) showed no differences in their IgG responses or T-cell responses to non-spike antigens.

3.3 Discussion

COVID-19 vaccines provide substantial protection against disease, yet breakthrough infections have remained common (Hacisuleyman et al., 2021; Kustin et al., 2021). This has led to considerable interest in individuals who appear to have no known SARS-CoV-2 infection despite repeated exposure. In this study, I found that the majority of participants who reported no prior infection exhibited immunological evidence compatible with asymptomatic SARS-CoV-2 exposure in both blood and nasal fluid.

Participants with no known infection showed systemic and nasal IgG responses to SARS-CoV-2 spike due to vaccination, with levels that were not significantly different to those observed in participants with recent infection. One-third of the NKI group also had detectable IgG to nucleocapsid in both systemic and nasal compartments, and this proportion almost doubled when nasal IgA to N or T-cell responses to non-spike antigens were examined. These findings suggest that the NKI group may have robust vaccine-induced anti-

spike responses that protect them from symptomatic infection, but many were likely to have experienced asymptomatic SARS-CoV-2 infection.

Interpretation of nasal antibody responses is confounded by the potential contribution of serum transudation. I observed a moderate correlation between S-specific IgG antibodies in the blood and nasal fluid within the NKI group, suggesting that systemic immunity may partially contribute to mucosal antibody levels. This is consistent with previous reports showing that intramuscular COVID-19 vaccines can elicit detectable mucosal immune responses (Sheikh-Mohamed et al., 2022; Stolovich-Rain et al., 2023). However, the induction of mucosal IgA following vaccination is generally limited (Liew et al., 2023; Sheikh-Mohamed et al., 2022). In line with this, I found a weaker correlation between systemic IgG and nasal IgA, together with stronger correlations among nasal antibody isotypes. These patterns indicate that the nasal IgA observed in the NKI group is driven predominantly by local mucosal immune processes or prior infection rather than systemic antibody levels.

It is possible that HCWs who showed antibody responses to SARS-CoV-2 non-spike proteins have pre-existing immunity to seasonal HCoVs rather than undetected SARS-CoV-2 infection. However, this seems less likely because the antibody levels to HCoV S did not differ between the NKI and DI groups, although they were higher than the UE group. I also found a stronger correlation between SARS-CoV-2 S- and HKU1 S- or OC43 S-specific antibodies in the DI group. I speculated that the correlation for antibodies to betacoronaviruses may indicate a boosting effect due to infection rather than indicating increased susceptibility to SARS-CoV-2, as shown in another study (Woudenberg et al., 2021). In the present study, I am unable to draw conclusions regarding the implication of cross-reactive responses because longitudinal data were not available. However, previous

reports have shown that pre-existing OC43 S-specific antibodies and alphacoronavirus N-specific antibodies were associated with an increased risk of severe COVID-19 (Focosi et al., 2021; Oppenheimer, 2025; Wratil et al., 2021). Conversely, other study suggested that pre-existing HCoV N-specific IgA and IgG were associated with protection against symptomatic infection (Ortega et al., 2021) or relative ratio of IgGs to all four HCoVs were indicators of neutralisation (Galipeau et al., 2021). Another showed no associations between cross-reactive immune responses to HCoVs and COVID-19 infection (Imai et al., 2021; Sermet-Gaudelus et al., 2021).

Similar to antibody responses, I did not observe differences between ELISpot T-cell responses to SARS-CoV-2 S between the NKI and DI groups, indicating that spike-specific antibody and T-cell responses reflect vaccination status rather than a distinguishing marker of breakthrough infection. Early T-cell proliferation has been associated with less severe disease (Chandran et al., 2022), especially $CD8^+$ T-cell responses to overlapping peptides from S, M and N (Sekine et al., 2020). In this study, proliferative T-cell responses against spike following vaccination and breakthrough infection were modest and predominantly $CD8^+$ T cells. Compared with the UE group, the NKI group had higher proliferative S-specific $CD4^+$ and $CD8^+$ T-cell responses, whereas the DI group showed higher proliferative $CD8^+$ T-cell responses than the UE group. Proliferative $CD4^+$ T-cell responses were not robustly detected in the DI group, potentially reflecting differences in proliferative kinetics between $CD8^+$ and $CD4^+$ T cells, with $CD8^+$ T cells expanding more rapidly (Foulds et al., 2002), which may influence their relative detectability following recent infection.

T-cell responses to non-structural proteins were limited in the NKI group, in contrast to highly exposed seronegative individuals, where strong non-structural protein-specific T-cell responses have been associated with protection from detectable SARS-CoV-2 infection

(Swadling et al., 2022). The NKI group in this study differs from the seronegative group in Swadling *et al*.'s study as participants here were not as closely monitored. Participants in the NKI group may have been infected but missed the viral detection window, while others may have had lower exposure to viruses. For example, some participants in the HCW study had working roles that allowed them to work from home, and there were a few individuals who continued to restrict their social contacts well beyond the government's restrictions. Nevertheless, in this study, I did not observe differences in immune responses between those with patient-facing roles and those without. Another difference in this study is that the peptide pools used were 16-18-mers with 10-mers overlapping, whereas Swadling *et al*. used 15-mers peptides (Supplementary Table 10). Only four out of the nine NSP peptides immunogenic to CD4+ T-cells and three out of six peptides immunogenic to CD8+ T-cells were common between this study and Swadling's. Swadling *et al*. also plated 400,000 PBMCs per well, compared with 200,000 PBMCs per well in my assay. As ELISpot sensitivity is influenced by the absolute cell input per well, this difference may have reduced my ability to detect low-frequency NSP-specific responses, even though responses are reported as SFU per 10^6 PBMCs.

To distinguish asymptomatic SARS-CoV-2 infection from potential cross-reactive responses to seasonal human coronaviruses, I evaluated multiple non-S specific immune parameters rather than relying on a single marker. The consistent presence of anti-N antibody responses across compartments in the DI group, particularly when accompanied by non-S T-cell reactivity, supports recent SARS-CoV-2 infection, as nucleocapsid is not included in spike-only vaccines they received. In contrast, participants with isolated non-S T-cell responses or single-compartment nucleocapsid antibody detection may reflect prior unrecognised SARS-CoV-2 exposure or cross-reactive immunity derived from seasonal coronaviruses. Non-S T-cell reactivity alone cannot definitively distinguish between SARS-

CoV-2 infection and cross-reactivity, as pre-existing T-cell responses to conserved coronavirus epitopes have been reported (Anderson et al., 2021; Grifoni et al., 2020; Le Bert et al., 2020). Anti-N IgG in the blood has been estimated to have a half-life of 85 days (Lumley et al., 2021), so individuals who had been infected months ago may not have detected anti-N IgG in their blood.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, sampling time points were heterogeneous both within and between study groups, which may have introduced variability in measured immune responses. Second, the number of samples that passed quality control for cellular assays was reduced due to a freezer break-down, limiting statistical power. As a result, certain findings should be interpreted cautiously and warrant confirmation in larger datasets. In addition, peptide pools used for T-cell stimulation were optimised for $CD4^+$ T-cells, rather than $CD8^+$ T-cells. $CD8^+$ T-cells bind more effectively to shorter peptides, 9-10-mers, so the $CD8^+$ T-cell responses measured in this study may have been underestimated.

Beyond addressing the limitations mentioned before, future work could include evaluating the durability of immune responses in the NKI group to distinguish long-lasting vaccine-induced immunity from responses generated by unrecognised asymptomatic infection. Additional studies examining T-cell responses to HCoVs and incorporating HLA typing would also be informative. Prior work has shown that T-cell responses to seasonal coronaviruses such as OC43 and HKU1, as well as the HLA-B*15:01 allele, are associated with asymptomatic SARS-CoV-2 infection (Augusto et al., 2023). Memory B-cell responses may likewise represent an important correlate of protection, as lower RBD-specific memory

B-cell frequencies have been linked to Delta breakthrough infection (Tay et al., 2022). However, I was unable to assess memory B-cells or detailed T-cell phenotypes in this study due to the limited availability of PBMC samples.

Conclusion

Overall, these findings demonstrate that a substantial proportion of individuals who self-reported no prior SARS-CoV-2 infection in the UK cohort had robust immune responses induced by vaccination and may have experienced asymptomatic infection. This observation shows the heterogeneity of vaccine responsiveness and the widespread viral transmission during the pandemics. Whether any participants in the NKI group possessed immune profiles that conferred true protection from infection remains to be investigated further.

4 Pre-second dose blood proteomic signatures associated with T-cell responsiveness to mRNA COVID-19 vaccines and comparison of post-vaccination immunophenotypic characterisation between T-cell response and non-response groups

4.1 Introduction

Protective immunity following vaccination depends on the capacity of the immune system to mount robust cellular and humoral responses. These responses are often blunted or functionally impaired with increasing age and in the presence of chronic disease, such as CMV and HIV, placing them at greater risk of severe infection and poor outcomes (Crooke et al., 2019; Murdaca et al., 2023; Palacios-Pedrero et al., 2022; Rees-Spear & McCoy, 2021). Heterogeneity in vaccine responsiveness is observed even among healthy individuals, with important implications for public and individual health. Despite this heterogeneity in healthy individuals, there remains a lack of simple, scalable clinical tools capable of identifying who will mount an effective immune response to vaccination.

The immune system is composed of multiple interacting cell populations whose collective balance determines systemic immune responses. Studies in healthy individuals have demonstrated that interindividual differences in immune responsiveness are driven by coordinated patterns of immune cell composition rather than by isolated cell types (Brodin et al., 2015; Kaczorowski et al., 2017; Tsang et al., 2014). Immune variation arises from both heritable and environmental influences, with longitudinal studies of twins revealing increasing divergence in immune profiles over time (Brodin et al., 2015). Comparisons of monozygotic twins discordant for CMV seropositivity have shown that chronic viral

infection can profoundly reshape the immune landscape, even in otherwise healthy individuals (Brodin et al., 2015).

Following the first dose of COVID-19 vaccination, most healthy vaccine recipients mounted detectable immune responses (Wei et al., 2022). In published studies, only a small proportion failed to develop measurable spike-specific T-cell responses, comprising approximately 6-8% of ChAdOx1 recipients and 4-5% of BNT162b2 recipients, and this proportion declined to below 1% following the second vaccine dose (Wei et al., 2022). In contrast, vaccine immunogenicity was highly variable and frequently attenuated in immunocompromised individuals, particularly those with primary or secondary antibody deficiency (Goodyear et al., 2024; Shields et al., 2022). Despite humoral responses being impaired, spike-specific T-cell responses were more frequently detectable, underscoring the importance of additional vaccine doses to enhance antibody responses and overall immunity in this population (Goodyear et al., 2024; Shields et al., 2022).

Pre-vaccination proteomic markers have previously been used to predict antibody responses following two doses of the inactivated COVID-19 vaccine BBIBP-CorV, as well as the magnitude and durability of humoral immune responses up to six months after a third dose of mRNA vaccines (Hickey et al., 2025; Hu et al., 2024). Pre-vaccination proteomic signatures, particularly those reflecting inflammatory and innate immune pathways, are predictive of the magnitude and durability of antibody responses to COVID-19 vaccination, supporting the use of blood-based protein profiling to identify individuals at risk of poor vaccine responsiveness (Hickey et al., 2025; Hu et al., 2024). In contrast, comparable protein-based predictors of vaccine-induced T-cell responses remain less well defined.

T-cells play important role in protection against SARS-CoV-2 by limiting disease severity, especially when antibody responses wane or viral variants evade neutralisation. A previous study showed that lower $CD4^+$ and $CD8^+$ T-cells, along with antibodies, have been correlated with Delta breakthrough infection (Neale et al., 2023). Severe COVID-19 is associated with impaired type I IFN signalling, lymphopenia, and failure of naïve T-cells to differentiate into effector and memory cells, compromising viral control (Dan et al., 2021; Diao et al., 2020; Hadjadj et al., 2020). Unlike anti-spike IgG which declined six months after second and third dose of BNT162b2 (Moore et al., 2023), T-cell and B-cell responses were more long-lived than antibodies (Bilich et al., 2021; Moore et al., 2023; Rydyznski Moderbacher et al., 2020), providing longer protection against COVID-19. In addition, T-cell responses generated after vaccinations has been shown to recognise SARS-CoV-2 variants and can potentially offer cross-protection (Tarke et al., 2022), which is important for controlling infection from highly mutated viruses.

I hypothesised that 1) baseline circulating immune markers reflecting immune ageing and chronic viral infection are associated with vaccine-induced T-cell responsiveness; and 2) T-cell non-response group display immunophenotypic features consistent with immune senescence or functional impairment, whereas response group exhibit features of preserved immune competence and regulated activation.

The first aim of this chapter was to identify baseline serological and demographic factors associated with vaccine-induced T-cell responsiveness, including age, sex, and CMV-specific IgG, and to integrate these variables into a proof-of-concept immune competence score. Such a tool demonstrates the feasibility of capturing inter-individual heterogeneity in cellular immune responses, with potential relevance of optimising vaccination strategies at the population level. The second aim was to characterise immunophenotypic features that

distinguish T-cell response group from non-response group among healthy UK healthcare workers following two doses of COVID-19 mRNA vaccination, and to assess whether these features are similarly associated with T-cell responsiveness in an immunocompromised cohort with primary or secondary antibody deficiency receiving immunoglobulin replacement therapy after three vaccine doses.

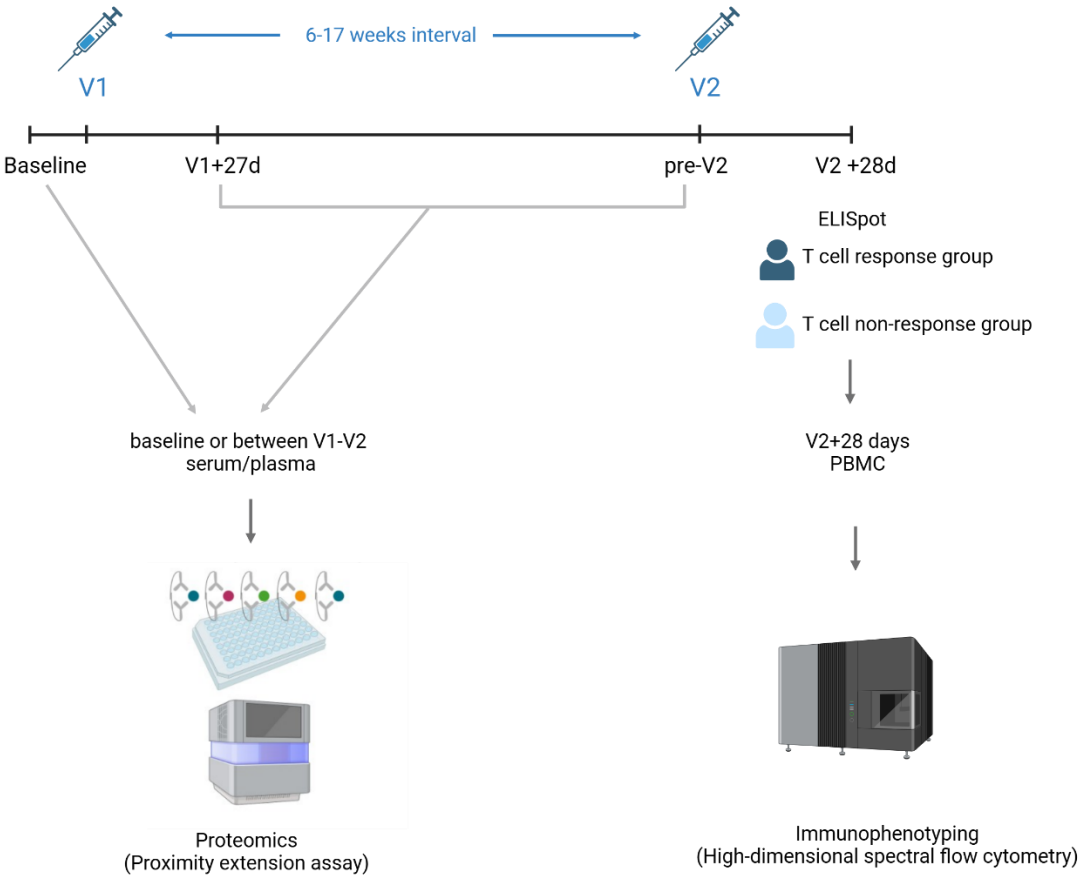

Figure 4-1 Study overview

Serum and plasma collected prior to the second dose of BNT162b2 COVID-19 vaccination from SARS-CoV-2 infection-naïve healthcare workers were analysed using Olink proteomics. Immunophenotyping was performed by high-dimensional spectral flow cytometry one month after the second dose. T-cell responsiveness measured one month after the second dose was defined by IFN-γ ELISpot responses >50 spot-forming units per 1×10^6 cells. *Created with Biorender.com.*

4.2 Results

4.2.1 Study population

I selected 176 archived serum and plasma samples from 143 healthcare workers (HCWs) across the five PITCH sites (Birmingham, Liverpool, Newcastle, Oxford, and Sheffield, UK). These samples were chosen specifically because T-cell data at V2+28 days were available, and therefore represent a discovery cohort rather than being randomised as population-representative. To reduce cohort heterogeneity, I restricted the cohort to participants who were SARS-CoV-2 infection-naïve and who had received the Pfizer BNT162b2 mRNA vaccine with the extended dosing interval (range approximately 6–17 weeks) (Payne et al., 2021).

Samples included baseline serum or plasma (n=111), pre-second dose samples (n=30). There are additional samples (n=2) from timepoint V2+31 days to support paired serum and plasma comparison analysis (Table 4-1). Participants were 22-65 years old and 74% (106/143) were female (Table 4-1). 37% (53/143) of participants had IgG to cytomegalovirus (CMV). One month after receiving two doses of mRNA COVID-19 vaccine, 78.3% (112/143) participants had measurable T-cell responses (>50 spot forming units per 1 million PBMCs) to SARS-CoV-2 spike, as assessed by IFNγ ELISpot. 87/143 had their anti-S IgG already measured and all participants were seropositive (Supplementary Figure 6).

Table 4-1 Characteristics of study population

	Study participants (n=143)
Age, year (range, mean ± SD)	22-65 years, 35.72±11.27
Sex, n (%)	
Male	37 (26%)
Female	106 (74%)
V1-V2 interval, days	45-124 days
T-cell response group at V2+28 days[$]	
Response group	112 (78.3%)
Non-Response group	31 (21.7%)
IgG to SARS-CoV-2 S	
Positive	87 (60.8%)
Negative	0 (0%)
Unknown	56 (39.2%)
IgG to CMV	
Positive	53 (37.1%)
Negative, including borderline	90 (62.9%)
Collection sites	
Birmingham	42 (29%)
Liverpool	15 (10%)
Newcastle	27 (19%)
Oxford	47 (33%)
Sheffield	12 (8.4%)
Collection timepoint	
Baseline	111 (78%)
Between V1 and V2	30 (21%)
V2+31 days	2 (1.4%)
Sample type	
Plasma only	68 (48%)
Serum only	42 (29%)
Paired plasma and serum	33 (23%)

[$]Cut-off for T-cell response group is >50 spot forming units/million PBMCs

4.2.2 Serum has higher protein expression than EDTA plasma

A mixture of serum and plasma samples was included in this analysis because EDTA plasma was not collected at pre-V2 at one site, so including the dataset from plasma and serum maximised cohort inclusion.

Human serum and plasma are derived from whole blood that has been processed differently. Serum is collected after the coagulation process and centrifugation, whereas plasma is separated from blood cells using an anticoagulant and centrifugation. During the coagulation process, platelets release pro-inflammatory mediators and reduced levels of coagulation factors compared with plasma (Patel & Naik, 2020; Tvedt et al., 2015). Given these known differences, a direct comparison between serum and plasma was performed to characterise systematic effects associated with sample type.

Protein expression measured using the Olink Inflammation panel was first compared between serum and plasma. Paired serum-plasma samples were available for 32 participants and a total of 364 proteins were detected across all samples. Principal component (PC) analysis demonstrated partial overlap between serum and plasma samples, with no clear separation along either PC1 or PC2 (Figure 4-2). PC1 and PC2 explained 36.5% and 11.8% of the total variance, respectively. The overall proteomic profile remained broadly comparable although some proteins differed significantly with greater inter-individual variability observed in plasma than in serum.

Correlation analysis showed that protein expression levels measured in serum and plasma were moderately to weakly correlated overall. Seventy-two proteins were strongly correlated (Spearman's $r>0.7$, $p<0.05$), 148 proteins were moderately correlated (Spearman's

0.4<r<0.7, p<0.05), 19 proteins were weakly correlated (Spearman's r<0.4, p<0.05) and 126 proteins showed no significant correlation (Spearman's p≥0.05) (Supplementary Table 11).

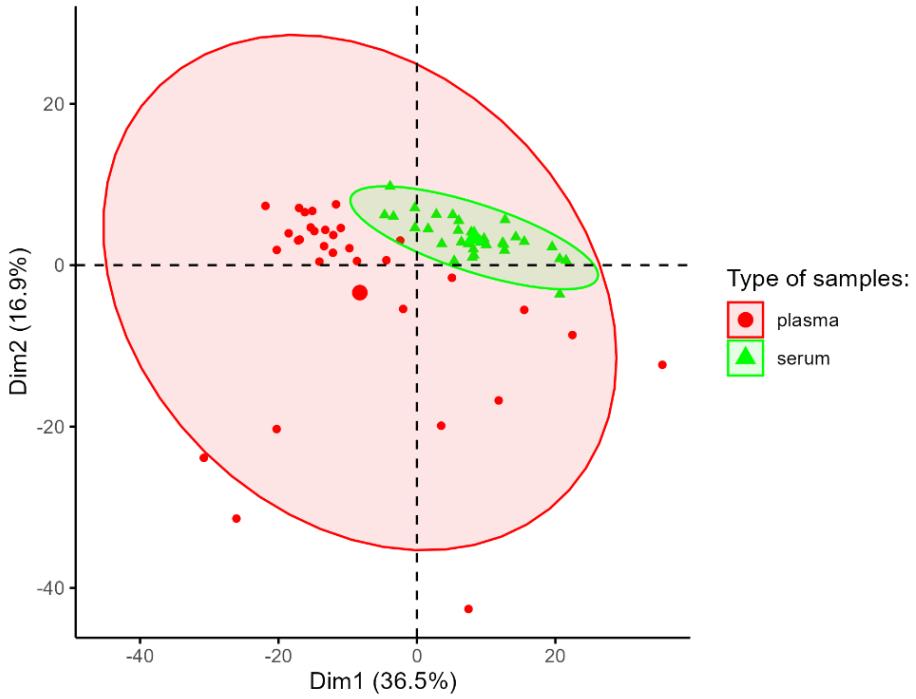

Figure 4-2 Principal component analysis of 364 protein expression in paired serum and plasma samples (n=32)

Principal component analysis (PCA) was performed on normalised $\log_2$ NPX values from 364 proteins to evaluate global differences in protein expression profiles between serum and plasma samples. Each point represents an individual sample, with serum and plasma samples from the same individual analysed as paired observations. Serum samples are shown in green and plasma samples in red. The first two principal components (PC1 and PC2) are shown and together explain percentage of the total variance.

Following the global comparison by PCA, univariable analysis was performed to identify individual proteins that differed significantly between plasma and serum. Using a threshold of absolute $\log_2$ fold change > 1 and adjusted p-value < 0.05, 73 proteins were differentially expressed between plasma and serum (Figure 4-3). Of these, 69 proteins showed higher expression in serum, while 4 proteins were significantly more abundant in plasma. The

largest differences were observed for EGF (log_2 fold change = 4.90), TIMP3 (log_2 fold change = 3.55), MPIG6B (log_2 fold change = 3.54), ANGPT1 (log_2 fold change = 3.49), and CD40LG (log_2 fold change = 3.35), all of which were higher in serum (Supplementary Table 11). In contrast, F2R (log_2 fold change = -2.56) and GMPR (log_2 fold change = -1.73) showed the greatest enrichment in plasma. Two proteins, CXCL17 and CLEC4D, despite their difference in expression level, were strongly correlated (Spearman's $r>0.7$, $p<0.05$), reflecting consistency between plasma and serum across individuals.

Pathway enrichment analysis of the 73 differentially expressed proteins between plasma and serum identified significant overrepresentation of Gene Ontology biological processes related to signalling and cell migration (Figure 4-4). The most significantly enriched terms included positive regulation of the mitogen-activated protein kinase (MAPK) cascade and multiple chemotaxis-related processes, including chemotaxis, cell chemotaxis, leukocyte chemotaxis, myeloid leukocyte migration, and taxis. In addition, several closely related phosphorylation-associated terms were enriched, including positive regulation of peptidyl-tyrosine phosphorylation, regulation of peptidyl-tyrosine phosphorylation, and peptidyl-tyrosine phosphorylation. Enriched terms are shown ordered by adjusted p-value.

These findings showed the characteristics of biomaterial-associated effects to guide subsequent analysis. Biomaterial type (serum versus plasma) was therefore included as a random effect in multivariable logistic regression analyses to account for biomaterial-dependent variation when identifying proteins that were associated with vaccine-induced T-cell responsiveness.

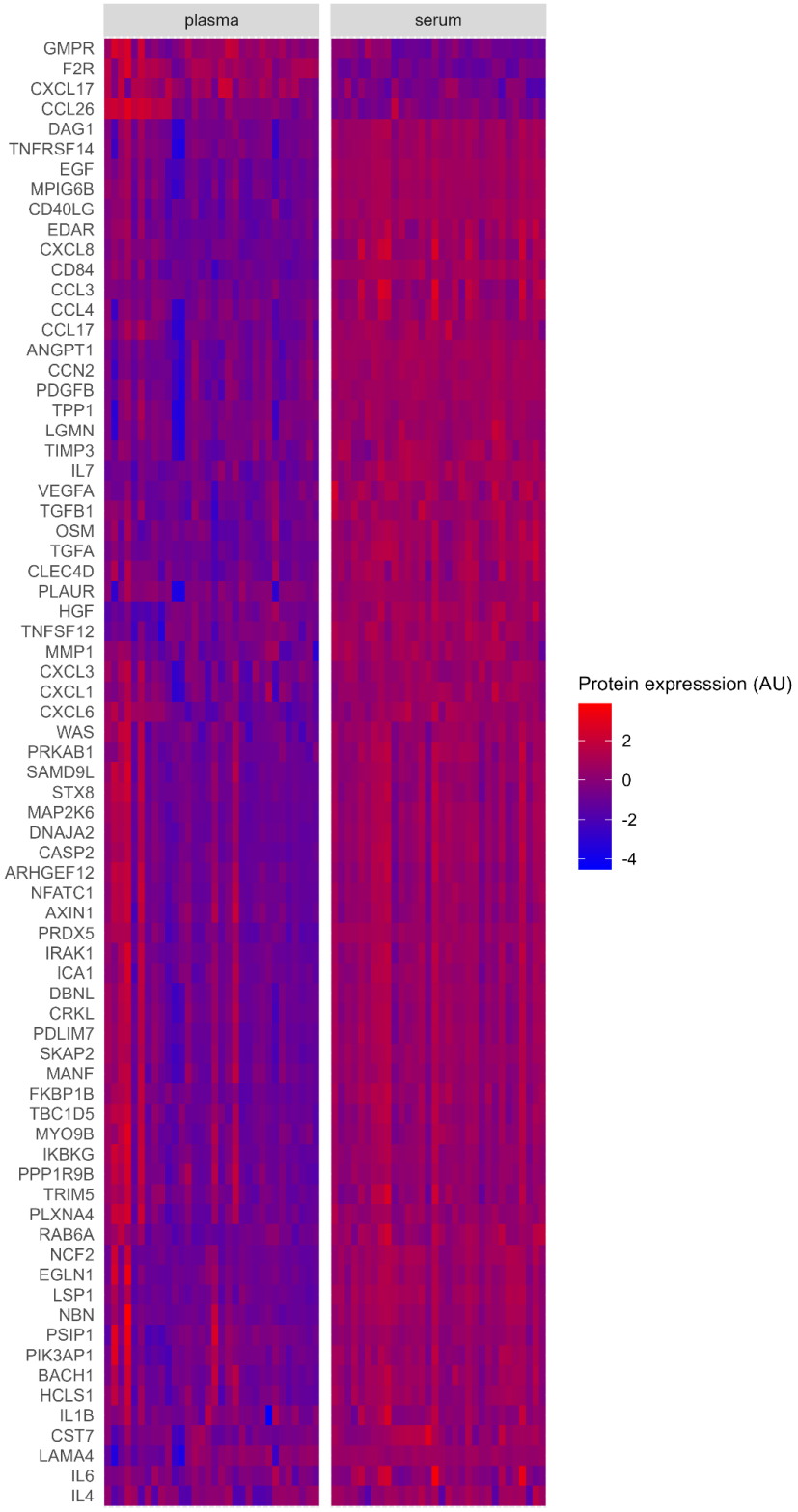

Figure 4-3 Heatmap of differentially expressed proteins in plasma and serum

Significantly different proteins (abs(log2 fold change)> 1, adjusted p<0.05) between paired plasma and serum (n=32) are ploted using heatmap. Each cell represents protein expression (arbitary unit - AU) from each sample. Comparison between two groups was measured using log2 fold change difference and p-value was adjusted using False Discovery Rate (FDR) method. Red indicates high protein expression and blue indicates low protein expression.

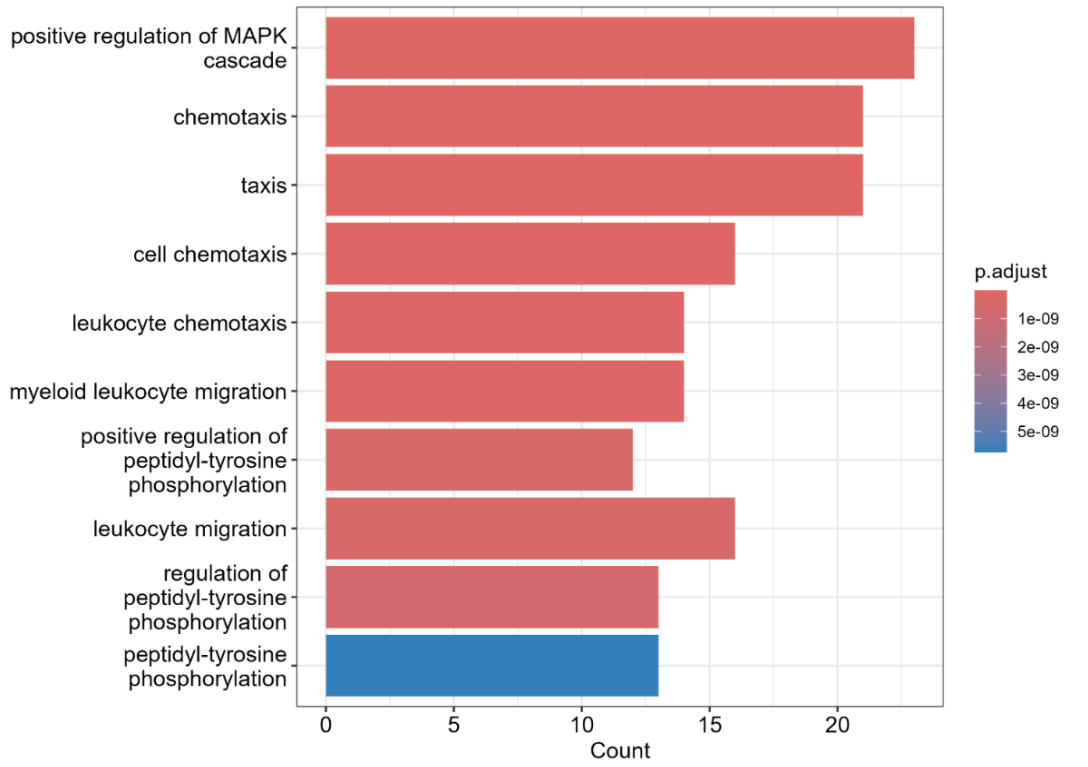

Figure 4-4 Gene Ontology pathway enrichment analysis of proteins differentially expressed between plasma and serum

Proteins that were significantly different between paired plasma and serum samples (n = 32; absolute $\log_2$ fold change > 1, adjusted p-value < 0.05) were assessed using Gene Ontology (GO) pathway enrichment analysis to identify biological processes overrepresented among the differentially expressed proteins.

4.2.3 Selecting features associated with T-cell responsiveness

Having accounted for sample type effects and established overall serum and plasma comparability, the analysis next focused on identifying baseline protein biomarkers associated with T-cell vaccine responsiveness.

The majority of samples included in the model training and test dataset were collected at baseline (n=109), with a smaller subset collected between the first and second vaccine doses (V1 and V2, n=32). Only samples that were collected approximately one month after the

first vaccine dose were included to account for the vaccine effect after vaccination. Paired longitudinal proteomic data comparing baseline and V1-V2 samples from the same individuals were not available. Therefore, PCA was conducted using cross-sectional samples from different individuals. The PCA showed broadly similar proteomic distributions between the timepoints, indicated by the overlapping clusters (Figure 4-5). To account for potential variability, the sample timepoint was also incorporated as a random effect in the model.

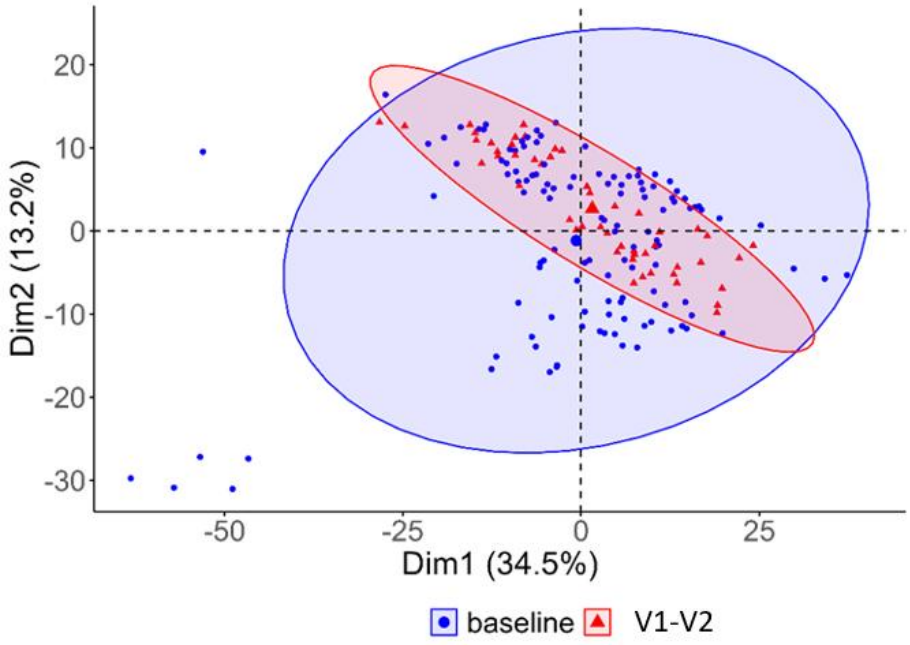

Figure 4-5 Principal component analysis of 364 protein expression in baseline and V1-V2 samples

Principal component analysis was performed on normalised log_2 NPX values for 364 proteins to evaluate global differences in protein expression profiles between samples collected at baseline (n=109) and those collected between the first and second vaccine doses (V1-V2) (n=32). Each point represents an individual sample. Baseline samples are shown in purple and V1-V2 samples are in red. The first two principal components (PC1 and PC2) are shown and together explain percentage of the total variance.

Univariate comparisons between the T cell response group and non-response groups were performed using the Wilcoxon rank-sum test. After adjustment for multiple testing using the False Discovery Rate (FDR), no proteins were significantly differentially expressed between the groups (absolute $\log_2$ fold change > 1 and adjusted p-value < 0.05) (Figure 4-6). Before multiple testing correction, there are 24 proteins that were significantly different between groups (Supplementary Table 12).

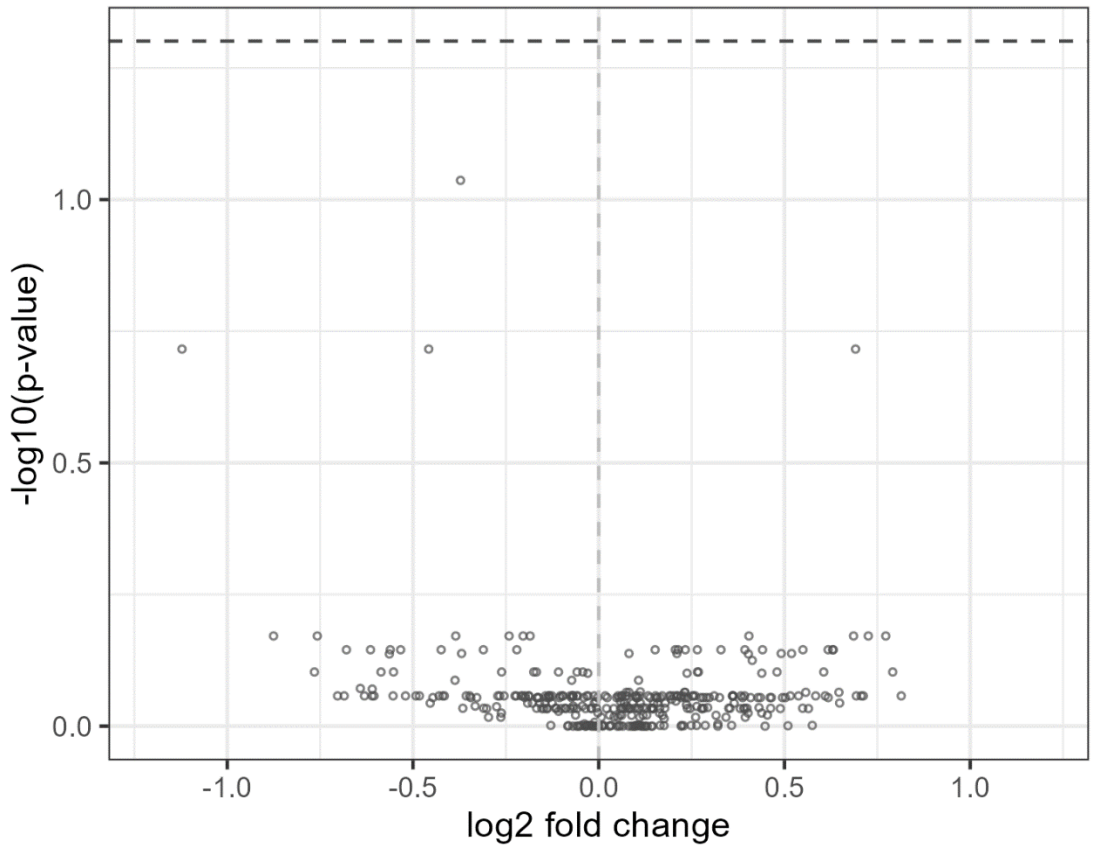

Figure 4-6 Comparison of protein expression between T-cell response and non-response groups
Difference in protein expression between T-cell response and non-response groups are shown in a volcano plot. Each dot represents the log2 fold change in protein expression between the two groups. Vertical dashed line indicates p-values cut-off adjusted for multiple testing using False Discovery Rate (FDR) method. Significantly different proteins is defined as abs(log2 fold change)> 1, adjusted p<0.05.

The dataset was split into a training set (80%, n = 117) and an independent validation set (20%, n = 34). Using the training dataset, multivariate analysis using least absolute shrinkage and selection operator (LASSO) regression were used to 364 proteins measured prior to the second dose of the BNT162b2 vaccine. Following feature selection, five proteins were identified as significant markers associated with T-cell responsiveness at V2+28 days: eukaryotic translation initiation factor 5A (eIF5A), interleukin 1 receptor like 2 (IL1RL2), collagen type IX alpha 1 chain (COL9A1), matrix metallopeptidase 1 (MMP-1) and Src kinase associated phosphoprotein 2 (SKAP2) (Figure 4-7). With the exception of MMP-1 and SKAP2 which were moderately correlated (Spearman's $r=0.57$, $P<0.001$), the other proteins were either weakly correlated or uncorrelated with one another, indicating limited collinearity among selected markers (Supplementary Figure 7).

Since the selected proteins may be influenced by the ratio of the number of samples to the number of proteins (Lee et al., 2024), the analysis was repeated using the full dataset for model training. LASSO analysis performed using the full dataset for model training identified eight proteins, including the five proteins previously selected using 80% of the dataset for training, as well as three additional proteins: Dipeptidyl Peptidase Like 10 (DPP10), Transforming Growth Factor Alpha (TGFA) and Protease, Serine 8 (PRSS8). Because the five proteins were consistently selected across both analyses, subsequent analyses were performed using the training and validation sets.

Following protein selection, a mixed-effects logistic regression model was fitted including the five reproducibly selected proteins, age, sex, and CMV IgG serostatus as fixed effects. Random intercepts were also included to account for inter-individual variability, sample type, and sample collection timepoint.

Increasing age was associated with higher odds of being a T-cell response group (OR=1.02 per year increase, 95% CI: 1.013-1.018). Higher levels of pre-V2 eIF5A (OR=2.758 per NPX unit increase, 95% CI: 2.751-2.764) and COL9A1 (OR=3.262 per NPX unit increase, 95% CI: 3.254-3.270) were associated with increased odds of T-cell responsiveness. In contrast, male sex (OR=0.079 per NPX unit increase, 95% CI: 0.0791-0.0794), CMV-specific IgG serostatus (seropositive OR=0.536, 95% CI: 0.535-0.538) and higher pre-V2 levels of IL1RL2 (OR=0.466 per NPX unit increase, 95% CI: 0.465-0.467), MMP-1 (OR=0.600 per NPX unit increase, 95% CI: 0.599-0.602), and SKAP2 (OR=0.871 per NPX unit increase, 95% CI: 0.869-0.873) were associated with reduced odds of T-cell responsiveness (Figure 4-7, Supplementary Table 13).

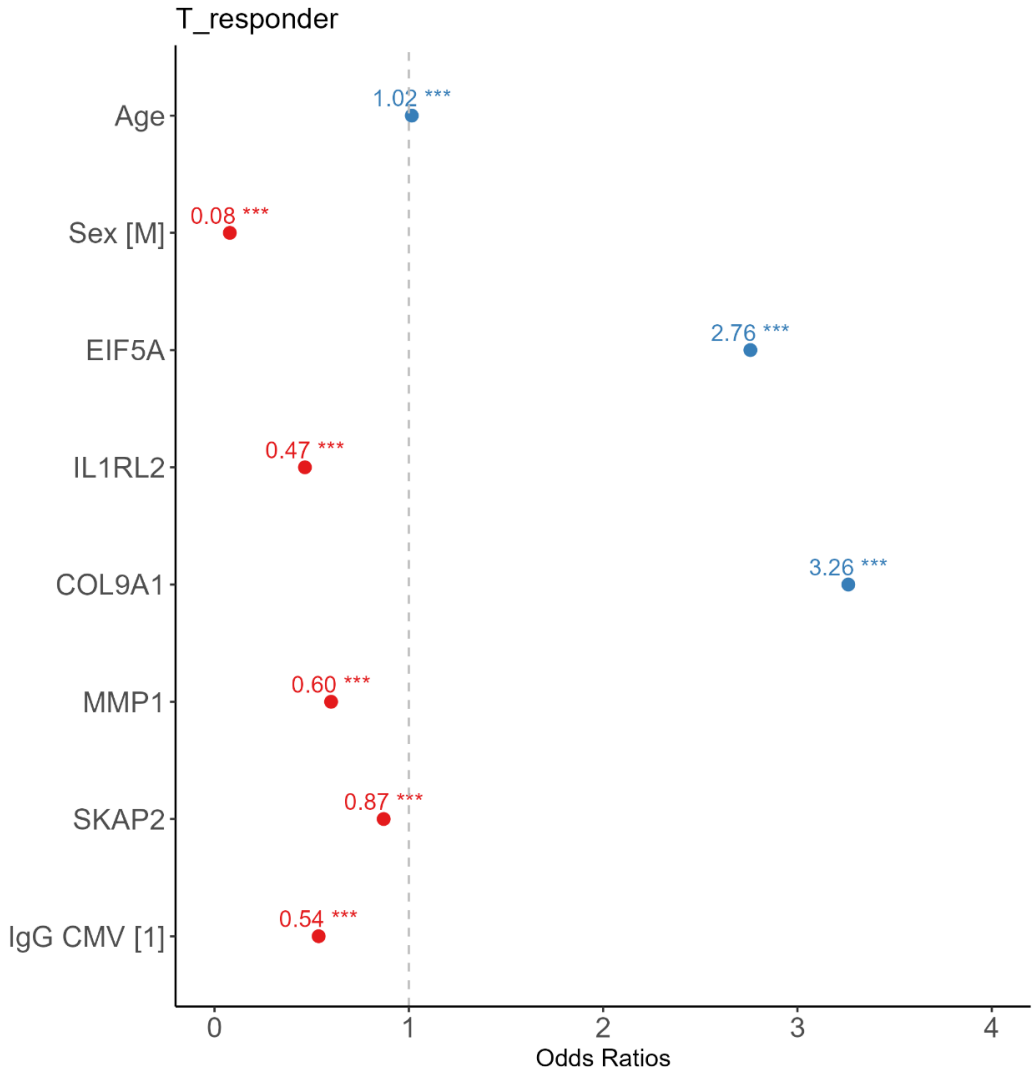

Figure 4-7 Proteomic and demographic markers associated with T-cell responsiveness after BNT162b2 COVID-19 vaccination

Proteomic and demographic markers associated with vaccine-induced T-cell responsiveness were identified using mixed-effects logistic regression with least absolute shrinkage and selection operator (LASSO), accounting for random effects: inter-individual variability, sample type and sample collection timepoint. Protein expression values are reported as NPX (normalised protein expression), which is a $\log_2$-transformed relative quantification; therefore, a one-unit increase in NPX corresponds to an approximate two-fold increase in protein abundance. *=p<0.05, **=p<0.01, ***=p<0.001.

Model discrimination was evaluated in the validation dataset (n=34). The model discrimination was assessed using the area under receiver operating characteristic curve,

which evaluate performance across classification thresholds. The model showed modest discrimination, with an AUC of 0.69 (95%CI: 0.48-0.89).

The probability of T-cell responsiveness, immunocompetence score, was then calculated for different combinations of demographic and proteomic features in the model (Table 4-2). This score translates the multivariable model into an interpretable metric that can compare immunological "fitness" across individuals. Protein expression levels were dichotomised at their median (low versus high expression) and participants were categorised into young (22-40 years) and older (41-65 years) age groups. The immunocompetence score for an older female aged 41-65 years, seronegative for CMV and with high levels of eIF5A and COL9A1 and low levels of IL1RL2, MMP-1 and SKAP2 was 99.9% (97.4-100.6%) (Table 4-2). In contrast, the immunocompetence score for a younger male, who was CMV-seropositive and had low levels of eIF5A and COL9A1, but high levels of IL1RL2, MMP-1 and SKAP2 was 27.5% (20.2-34.9%).

Table 4-2 The predicted immunocompetence (IC) score of T-cell responsiveness based on the fitted mixed-effect logistic regression model with LASSO selection

Scenario example	Age	Sex	CMV	EIF5A	COL9A1	IL1RL2	MMP1	SKAP2	Predicted IC score	95% CI
1	Y	M	+	L	L	H	H	H	0.275	0.202, 0.349
2	Y	M	-	L	L	H	H	H	0.279	0.205, 0.352
3	Y	F	+	L	L	H	H	H	0.415	0.334, 0.496
4	Y	F	-	L	L	H	H	H	0.828	0.765, 0.890
5	O	M	+	H	H	L	L	L	0.899	0.850, 0.949
6	O	M	-	H	H	L	L	L	0.934	0.894, 0.975
7	O	F	+	H	H	L	L	L	0.964	0.933, 0.995
8	O	F	-	H	H	L	L	L	0.990	0.974, 1.006

Legend. Y = younger adult (22–40 years); O = older adult (41-65 years). M = male; F = female. CMV: + = seropositive, − = seronegative. H = protein level > cohort median; L = ≤ cohort median (EIF5A, COL9A1, IL1RL2, MMP1, SKAP2). Predicted IC score = model probability (0–1) of being an ELISpot T-cell response group; 95% CI = 95% confidence interval.

4.2.4 Validating protein predictors using ELISA

To explore the translational potential of the identified protein predictors, I next evaluated whether they were measurable by ELISA. Previous studies have shown that COL9A1 and MMP-1, are secreted extracellular proteins and measurable in blood, whereas eIF5A and IL1RL2 are predominantly intracellular and have only been detected using highly sensitive platforms such as Olink (Karlsson, Zhang et al. 2021). SKAP2 is also an intracellular protein, but has been detected in circulation at low concentrations in blood at concentrations of 1.1 ng/mL using mass spectrometry (Karlsson, Zhang et al. 2021). Based on these considerations, I attempted to measure these proteins by sandwich ELISA in pre-V2 samples from T-cell non-response group (n=10) and T-cell response group (n=15).

Pre-V2 COL9A1, SKAP2 and MMP-1 were detectable in all plasma samples (100%, 25/25) by ELISA, whereas IL1RL2 and eIF5A were detectable in only 4% (1/25) and 20% (5/25) samples, respectively. There were no significant differences in COL9A1 or MMP-1 concentrations between T-cell non-response group and response group (COL9A1: median 8.8 ng/mL vs 11.6 ng/mL; MMP-1: median 2.0 ng/mL vs 2.2 ng/mL; $p \geq 0.05$ for both). In contrast, SKAP2 levels were significantly lower in response group (median 137.2 ng/mL) compared with non-response group (median 164.7 ng/mL; $p = 0.03$) (Figure 4-8). This observation is consistent with the earlier predictive modelling, which indicated that higher SKAP2 levels were associated with reduced odds of being a T-cell response group (Figure 4-7).

To assess cross-platform correlation, I examined the correlation between protein levels quantified by ELISA and by the Olink proteomic assay. SKAP2 concentrations measured by ELISA showed a moderate correlation with SKAP2 expression measured by Olink (Spearman's $r=0.5$, $p<0.01$) (Figure 4-9c). In contrast, no meaningful correlation was

observed for COL9A1 or MMP-1, indicating that these proteins may be less consistently quantified across assay platforms.

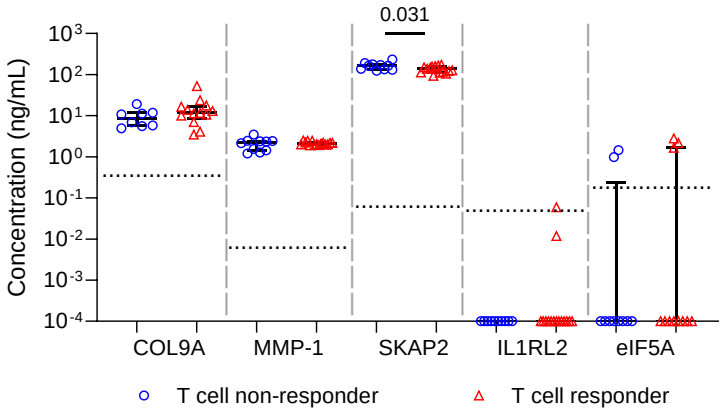

Figure 4-8 Levels of protein predictors of T-cell responsiveness as measured by ELISA

Plasma concentrations of the five protein predictors of T-cell responsiveness were quantified by ELISA in T-cell non-response group (n = 10) and response group (n = 15). Protein levels are shown with medians and interquartile range. Values plotted at 10^{-4} represent measurements below the assay's limit of detection (LOD). The LODs were: COL9A1 < 0.35 ng/mL, MMP-1 < 0.0058 ng/mL, SKAP2 < 0.06 ng/mL, IL1RL2 < 0.05 ng/mL, and eIF5A < 0.17 ng/mL. Statistical comparisons between groups were performed using the Wilcoxon rank-sum test.

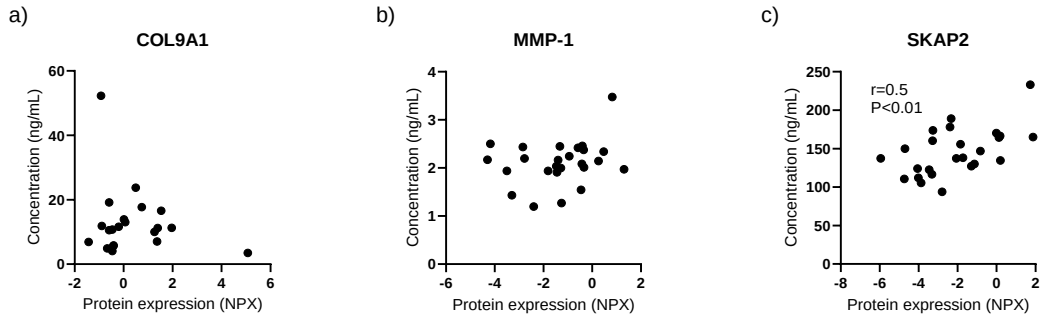

Figure 4-9 Correlation between protein levels measured by Olink® and ELISA

Plasma concentrations of a) COL9A1, b) MMP-1 and c) SKAP2 were quantified by ELISA and compared with corresponding NPX values measured by the Olink® platform. Correlations between Olink and ELISA measurements were assessed using Spearman's rank correlation test across all participants (T-cell response group, n = 15; non-response group, n = 10).

In the T-cell response group, I examined paired baseline and V2+28 days samples (n = 14) to assess the degree of variation in protein concentrations across timepoints (Figure 4-10). SKAP2 and MMP-1 showed modest decreases between the two measurements (median changes of -15.66 ng/mL and -0.22 ng/mL, respectively), while COL9A1 levels did not differ significantly between two timepoints.

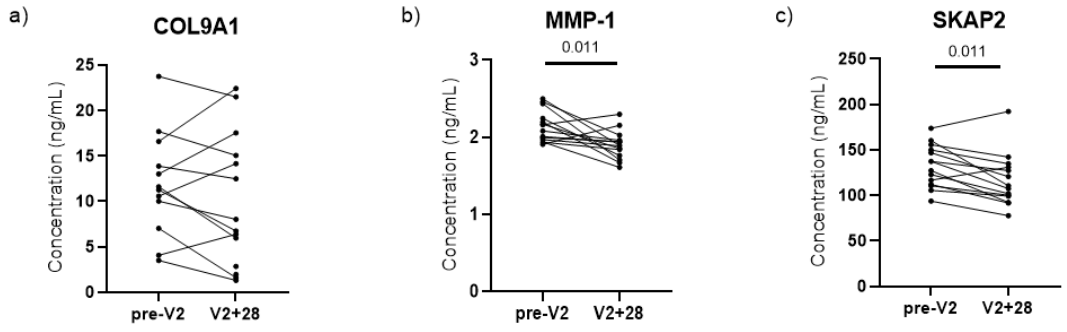

Figure 4-10 Comparison of protein concentrations before and one month after the second dose of BNT162b vaccine

Plasma concentrations of a) COL9A1, b) MMP-1 and c) SKAP2 were measured in paired samples (n=14) collected prior to V2 and one month after the second dose of BNT162b2. Differences between paired samples were analysed using paired t-tests.

4.2.5 Immunophenotyping of immune cell subsets in healthy individuals stratified by T-cell response status

To investigate whether specific immune cell phenotypes could help explain differences in T-cell responsiveness observed in the ELISpot assays, PBMCs collected one month after the second dose of BNT162b2 were analysed to compare immunophenotypic profiles between healthy T-cell response group and non-response group. Given the high dimensionality of the flow cytometry dataset and the scope of the study, a targeted manual gating strategy was employed to focus on T-cell features most relevant to vaccine-induced immunity.

Specifically, I assessed (1) T-cell differentiation states, reflecting the maturation status of $CD4^+$ and $CD8^+$ T cells; (2) regulatory T-cells ($CD4^+FoxP3^+CD25^+$), due to their capacity to suppress antigen-specific activation; and (3) markers of T-cell senescence and exhaustion, which are associated with impaired functional responses to vaccination.

I first compared T-cell differentiation states between T-cell response group and non-response group (Table 4-3). No significant differences were observed in $CD4^+$ T-cell differentiation subsets, including naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and EMRA ($CD45RA^+CCR7^-$) populations ($p \geq 0.05$). In contrast, response group exhibited higher frequencies of naïve $CD8^+$ T-cells (77.10%, IQR=61.80-82.20) than non-response group (48.1%, IQR=33.80-72.45), but lower effector memory $CD8^+$ T-cells ($CD8^+CD45RA^-CCR7^-$) (median=10.43%, IQR=6.24-22.15) compared with non-response group (median=21.20%, IQR=13.60-33.25).

Table 4-3 Comparison of T-cell subset proportions between healthy T-cell response group and non-response group at V2+28 days

$CD4^+$ T-cell	T-cell non-response group % $CD4^+$ (median [IQR])	T-cell response group % $CD4^+$ (median [IQR])	p-value*
central memory ($CD45RA^-$ $CCR7^+$)	21.80 [19.90-33.65]	19.35 [15.65-29.50]	0.264
effector memory ($CD45RA^-$ $CCR7^-$)	15.90 [11.20-25.00]	17.20 [11.03-24.18]	0.937
EMRA ($CD45RA^+$ $CCR7^-$)	0.99 [0.71-1.82]	1.56 [1.13-1.90]	0.128
Naive ($CD45RA^+$ $CCR7^+$)	59.30 [40.95-68.50]	60.40 [42.53-71.95]	0.687

$CD8^+$ T-cell	T-cell non-response group % $CD8^+$ (median [IQR])	T-cell response group % $CD8^+$ (median [IQR])	p-value*
central memory ($CD45RA^-$ $CCR7^+$)	14.90 [6.69-23.25]	7.95 [5.29-12.93]	0.072
effector memory ($CD45RA^-$ $CCR7^-$)	21.20 [13.60-33.25]	10.43 [6.24-22.15]	0.027
EMRA ($CD45RA^+$ $CCR7^-$)	8.66 [4.00-11.00]	3.52 [2.16-4.51]	0.068
Naive ($CD45RA^+$ $CCR7^+$)	48.1 [33.80-72.45]	77.10 [61.80-82.20]	0.02

* p-value was calculated using Wilcoxon rank-sum test.

Regulatory T-cells ($CD4^+FoxP3^+CD25^+$) were more abundant in T-cell response group (median=4.92%, IQR=4.10-6.21) than in non-response group (median=3.65%, IQR=2.93-4.54) (p=0.03), suggesting greater immunoregulatory activity in individuals with detectable T-cell responses (Figure 4-11).

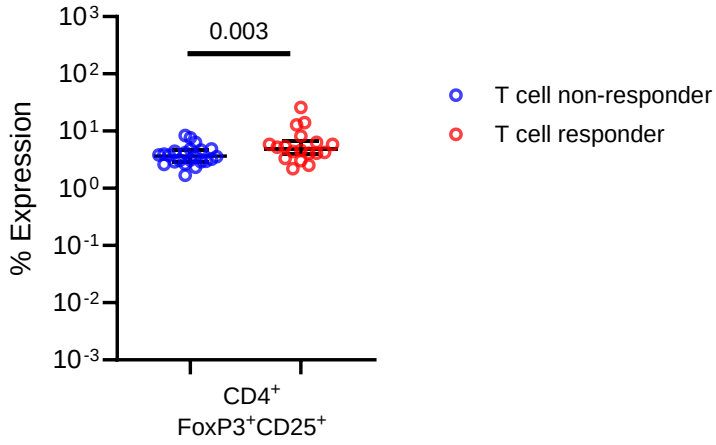

Figure 4-11 Comparison of regulatory T-cell frequencies between healthy T-cell non-response group and response group at V2+28 days

Peripheral blood mononuclear cells (PBMCs) were collected from healthy individuals classified as T-cell response group (n = 18) or non-response group (n = 23) based on SARS-CoV-2 spike–specific ELISpot responses one month after the second BNT162b2 dose. Regulatory T-cells were defined by $CD4^+FoxP3^+CD25^+$. Data are reported as medians with interquartile ranges (IQR), with individual samples shown. The comparison between groups was analysed using the Wilcoxon rank-sum test.

In contrast, markers of T-cell senescence were elevated in non-response group. Senescent $CD4^+$ T-cells ($CD4^+CD28^-CD57^+$) were more frequent in non-response group (median = 0.34%, IQR = 0.10–0.60) than in response group (median = 0.07%, IQR = 0.01–0.20) (Figure 4-12). Similarly, non-response group showed higher proportions of senescent $CD8^+$ T-cells ($CD8^+CD28^-CD57^+$) (median = 22.6%, IQR = 17.1–29.0) compared with response group (median = 13.2%, IQR = 9.5–18.6).

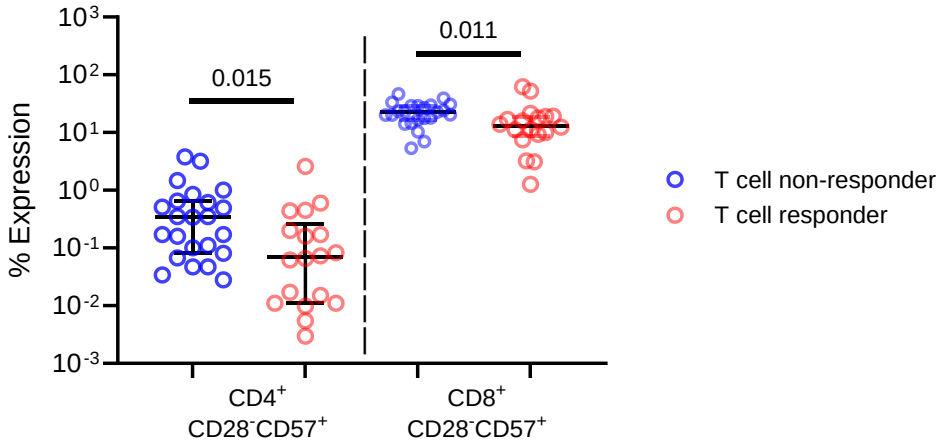

Figure 4-12 Comparison of senescent marker expression between healthy T-cell non-response group and response group at V2+28 days

Peripheral blood mononuclear cells (PBMCs) were collected from healthy individuals classified as T-cell response group (n = 18) or non-response group (n = 23) based on SARS-CoV-2 spike-specific ELISpot responses one month after the second BNT162b2 dose. Senescent $CD4^+$ and $CD8^+$ T-cells were defined by $CD28^-CD57^+$ co-expression. Data are reported as medians with interquartile ranges (IQR), with individual samples shown. The comparison between groups were analysed using the Wilcoxon rank-sum test.

T-cell exhaustion was assessed using expression of Programmed Death-1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), and T-cell immunoreceptor with immunoglobulin and tyrosine-based inhibitory motif domain (TIGIT) (Table 4-4). Among $CD4^+$ T-cells, response group exhibited higher frequencies of $CTLA4^+LAG3^+PD\text{-}1^+TIGIT^+$ cells (median=0.005, IQR=0.002-0.018) compared with non-response group (median=0.001, IQR=0.000-0.006). In contrast, non-response group demonstrated higher levels of several exhaustion-marker combinations within the $CD8^+$ T-cell compartment. Specifically, non-response group had increased frequencies of $CTLA4^-LAG3^-PD\text{-}1^-TIGIT^+$ (median=10.5%, IQR=6.8-18.2), $CTLA4^-LAG3^-PD\text{-}1^+TIGIT^+$ (median=3.0%, IQR=1.1-5.9), and $CTLA4^-LAG3^-PD\text{-}1^+TIGIT^-$ (median=1.1%,

IQR=0.5-2.4) $CD8^+$ T-cells compared with response group (5.9% [2.8–8.4], 0.8% [0.5–1.1], and 0.6% [0.3–1.2], respectively).

Table 4-4 Comparison of exhaustion marker expression on $CD4^+$ and $CD8^+$ T-cell between healthy T-cell non-response group and response group at V2+28 days

$CD4^+CD28^+$ T-cells								
				T-cell non-response group		**T-cell response group**		
CTLA4	LAG3	PD-1	TIGIT	Median	IQR	Median	IQR	p*
-	+	-	+	0.013	0.006-0.029	0.010	0.003-0.042	0.958
-	+	-	-	0.078	0.058-0.150	0.092	0.045-0.285	0.713
-	+	+	+	0.008	0.006-0.023	0.008	0.004-0.033	0.864
-	+	+	-	0.010	0.005-0.016	0.010	0.001-0.020	1.000
-	-	-	+	5.100	3.150-6.645	5.69	2.190-8.657	0.726
-	-	+	+	0.880	0.530-1.445	0.760	0.475-1.045	0.299
-	-	+	-	0.530	0.430-1.135	0.675	0.472-1.002	0.854
+	+	-	+	0.003	0.000-0.006	0.005	0.000-0.011	0.343
+	+	-	-	0.012	0.004-0.032	0.021	0.014-0.047	0.084
+	+	+	+	0.001	0.000-0.006	0.005	0.002-0.018	0.042
+	+	+	-	0.009	0.000-0.015	0.009	0.004-0.023	0.571
+	-	-	+	0.210	0.140-0.275	0.280	0.153-0.385	0.337
+	-	+	-	0.042	0.026-0.082	0.077	0.037-0.100	0.198
+	-	+	+	0.032	0.023-0.054	0.050	0.023-0.096	0.386
+	-	-	-	0.350	0.240-0.430	0.385	0.305-0.547	0.379

$CD8^+CD28^+$ T-cells								
				T-cell non-response group		**T-cell response group**		
CTLA4	LAG3	PD-1	TIGIT	Median	IQR	Median	IQR	p*
-	+	-	+	0.110	0.021-0.130	0.040	0.013-0.217	0.422
-	+	-	-	0.240	0.150-0.360	0.155	0.089-0.500	0.312
-	+	+	+	0.009	0.000-0.021	0.010	0.004-0.028	0.587
-	+	+	-	0.015	0.000-0.042	0.021	0.013-0.031	0.642
-	-	-	+	10.500	6.820-18.200	5.895	2.817-8.375	0.010
-	-	+	+	2.980	1.115-5.865	0.775	0.445-1.142	0.004
-	-	+	-	1.100	0.520-2.395	0.560	0.302-1.193	0.031
+	+	-	+	0.000	0.000-0.000	0.000	0.000-0.000	0.280
+	+	-	-	0.000	0.000-0.000	0.000	0.000-0.000	0.593
+	+	+	+	0.000	0.000-0.000	0.000	0.000-0.000	0.781
+	+	+	-	0.000	0.000-0.000	0.000	0.000-0.000	0.103
+	-	-	+	0.003	0.000-0.018	0.008	0.000-0.011	0.657
+	-	+	-	0.000	0.000-0.000	0.000	0.000-0.000	0.690
+	-	+	+	0.000	0.000-0.003	0.000	0.000-0.005	0.864
+	-	-	-	0.014	0.008-0.019	0.012	0.000-0.019	0.325

*p-value was calculated using Wilcoxon rank-sum test.

4.2.6 Immunophenotyping of immune cell subsets in immunocompromised individuals stratified by T-cell response status

Impaired vaccine responsiveness is common in individuals with primary or secondary antibody deficiencies. To extend these findings, samples from immunocompromised patients receiving immunoglobulin replacement therapy were analysed one month after their third COVID-19 vaccine dose (Supplementary Table 14). Including this cohort allowed assessment of whether immunological features associated with poor T-cell responsiveness in healthy individuals were also observed in a clinically vulnerable population who needed immunoglobulin replacement therapy.

T-cell differentiation states between T-cell response group (n=11) and non-response group (n=7) within the immunocompromised cohort did not differ significantly (Table 4-5). No significant differences were observed in $CD4^+$ T-cell and $CD8^+$ T-cell differentiation subsets, including naïve ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and EMRA ($CD45RA^+CCR7^-$) populations ($p \geq 0.05$).

Table 4-5 Comparison of T-cell subset proportions between immunocompromised T-cell response group and non-response group at V3+28 days

$CD4^+$ T-cell	T-cell non-response group % $CD4^+$ (median [IQR])	T-cell response group % $CD4^+$ (median [IQR])	p-value*
central memory ($CD45RA^-$ $CCR7^+$)	25.00 [16.40-29.80]	29.70 [21.90-36.30]	0.18
effector memory ($CD45RA^-$ $CCR7^-$)	25.40 [12.30-42.60]	24.80 [22.90-34.30]	0.86
EMRA ($CD45RA^+$ $CCR7^-$)	1.88 [0.55-2.62]	1.70 [1.33-2.97]	0.66
Naive ($CD45RA^+$ $CCR7^+$)	56.40 [26.30-70.90]	40.40 [21.60-58.60]	0.72

$CD8^+$ T-cell	T-cell non-response group % $CD8^+$ (median [IQR])	T-cell response group % $CD8^+$ (median [IQR])	p-value*
central memory ($CD45RA^-$ $CCR7^+$)	19.10 [11.30-24.70]	14.60 [7.29-35.30]	0.62
effector memory ($CD45RA^-$ $CCR7^-$)	27.10 [12.90-27.50]	22.70 [11.60-46.40]	1
EMRA ($CD45RA^+$ $CCR7^-$)	6.49 [3.16-17.60]	8.22 [4.74-11.40]	1
Naive ($CD45RA^+$ $CCR7^+$)	37.80 [32.30-74.30]	52.40 [17.90-68.90]	0.86

* p-value was calculated using Wilcoxon rank-sum test.

T cell subsets were compared between healthy individuals (at timepoint V2+28 days) and immunocompromised patients (at timepoint V3+28 days) (Table 4-6). Significant differences were observed in $CD4^+$ effector memory T-cells and $CD8^+$ naïve T-cells between groups. However, pairwise comparisons did not show statistically significant differences after adjustment for multiple testing. The median proportions of $CD4^+$ effector memory T-cells were lower in healthy individuals, T-cell non-response group = 15.90% [11.20-25.00%] and T-cell response group = 17.20% [11.03-24.18%], compared with immunocompromised patients, T-cell non-response group = 25.40% [12.30-42.60%] and T-cell response group = 24.80% [22.90-34.30%], although this difference was not statistically significant. In contrast, the median proportions of $CD8^+$ naïve T-cells, 77.10% [61.80-82.20%], was the highest in healthy T-cell response groups, but this difference was not statistically significant. No other T-cell subsets differed significantly between the groups.

Table 4-6 Comparison of T-cell subset proportions between healthy individuals at V2+28 days and immunocompromised T-cell response and non-response groups at V3+28 days

$CD4^+$ T-cell	Healthy individuals (V2+28 days)		Immunocompromised patients (V3+28 days)		
	T-cell non-response group % $CD4^+$ (median [IQR])	T-cell response group % $CD4^+$ (median [IQR])	T-cell non-response group % $CD4^+$ (median [IQR])	T-cell response group % $CD4^+$ (median [IQR])	p-value*
central memory ($CD45RA^-$ $CCR7^+$)	21.80 [19.90-33.65]	19.35 [15.65-29.50]	25.00 [16.40-29.80]	29.70 [21.90-36.30]	0.355
effector memory ($CD45RA^-$ $CCR7^-$)	15.90 [11.20-25.00]	17.20 [11.03-24.18]	25.40 [12.30-42.60]	24.80 [22.90-34.30]	0.048
EMRA ($CD45RA^+$ $CCR7^-$)	0.99 [0.71-1.82]	1.56 [1.13-1.90]	1.88 [0.55-2.62]	1.70 [1.33-2.97]	0.321
Naive ($CD45RA^+$ $CCR7^+$)	59.30 [40.95-68.50]	60.40 [42.53-71.95]	56.40 [26.30-70.90]	40.40 [21.60-58.60]	0.135

$CD8^+$ T-cell	Healthy individuals (V2+28 days)		Immunocompromised patients (V3+28 days)		
	T-cell non-response group % $CD8^+$ (median [IQR])	T-cell response group % $CD8^+$ (median [IQR])	T-cell non-response group % $CD8^+$ (median [IQR])	T-cell response group % $CD8^+$ (median [IQR])	p-value*
central memory ($CD45RA^-$ $CCR7^+$)	14.90 [6.69-23.25]	7.95 [5.29-12.93]	19.10 [11.30-24.70]	14.60 [7.29-35.30]	0.063
effector memory ($CD45RA^-$ $CCR7^-$)	21.20 [13.60-33.25]	10.43 [6.24-22.15]	27.10 [12.90-27.50]	22.70 [11.60-46.40]	0.093
EMRA ($CD45RA^+$ $CCR7^-$)	8.66 [4.00-11.00]	3.52 [2.16-4.51]	6.49 [3.16-17.60]	8.22 [4.74-11.40]	0.093
Naive ($CD45RA^+$ $CCR7^+$)	48.1 [33.80-72.45]	77.10 [61.80-82.20]	37.80 [32.30-74.30]	52.40 [17.90-68.90]	0.046

* p-value was calculated using Kruskal-Wallis test

In contrast to observations in healthy individuals, the frequency of regulatory T-cells ($CD4^+FoxP3^+CD25^+$) did not differ significantly between immunocompromised T-cell response group (median 3.12%, IQR 1.72–6.85) and non-response group (median 4.71%, IQR 4.24–8.65; p = 0.41) (Figure 4-13).

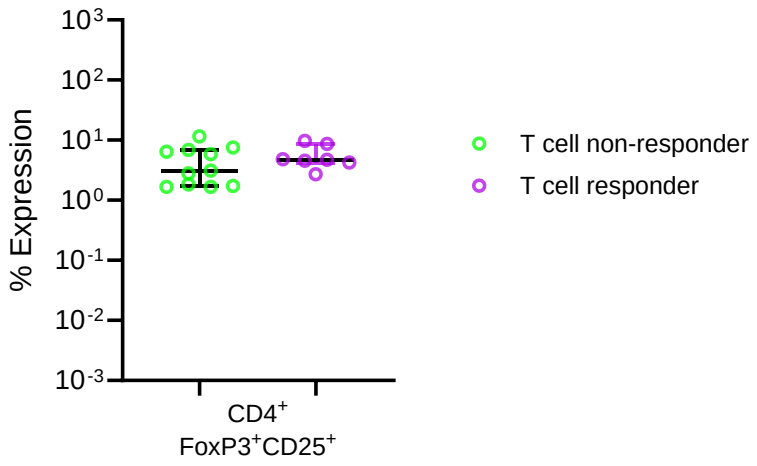

Figure 4-13 Comparison of regulatory T-cell frequencies between immunocompromised T-cell non-response group and response group at V3+28 days

Peripheral blood mononuclear cells (PBMCs) were collected from immunocompromised individuals classified as T-cell response group (n = 11) or non-response group (n = 7) based on SARS-CoV-2 spike-specific ELISpot responses one month after the third BNT162b2 dose. Regulatory T-cells were defined by $CD4^+FoxP3^+CD25^+$. Data are reported as medians with interquartile ranges (IQR), with individual samples shown. The comparison between groups were analysed using the Wilcoxon rank-sum test.

Similarly, levels of senescent T-cells did not differ significantly between immunocompromised individuals with and without detectable T-cell responses after the third dose (Figure 4-14). Among non-response group, the frequency of $CD4^+CD28^-CD57+$ T-cells was median 0.11% [0.07–0.94], and did not differ significantly to that observed in response group (median 0.17%, IQR 0.05–0.81). Likewise, the proportion of

$CD8^+CD28^-CD57^+$ T-cells did not differ significantly between non-response group (median 15.4%, IQR 8.71–37.3) and response group (median 18.8%, IQR 10.3–36.5).

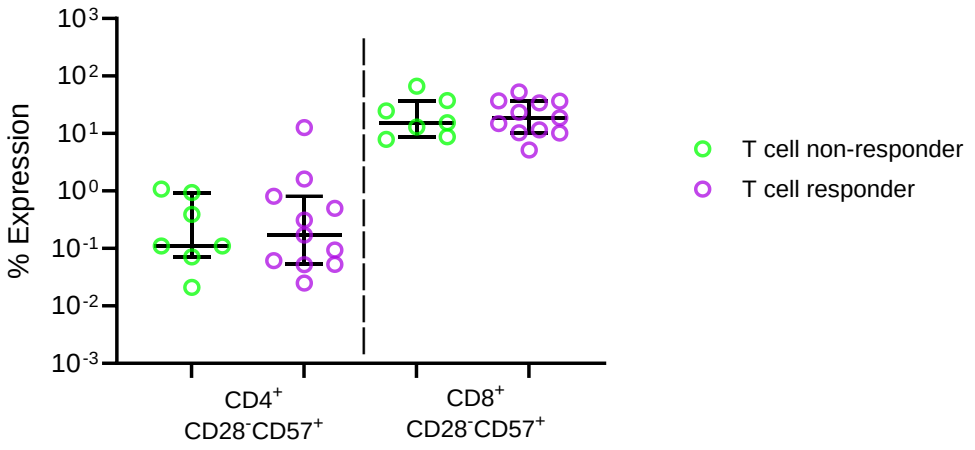

Figure 4-14 Comparison of senescent marker expression between immunocompromised T-cell non-response group and response group at V3+28 days

Peripheral blood mononuclear cells (PBMCs) were collected from immunocompromised individuals classified as T-cell response group (n = 11) or non-response group (n = 7) based on SARS-CoV-2 spike-specific ELISpot responses one month after the third BNT162b2 dose. Senescent $CD4^+$ and $CD8^+$ T-cells were defined by $CD28^-CD57^+$ co-expression. Data are reported as medians with interquartile ranges (IQR), with individual samples shown. The comparison between groups were analysed using the Wilcoxon rank-sum test.

T response group exhibited higher frequencies of $CD4^+$CTLA-4^+LAG-3^-PD-$1^+$$TIGIT^+$ cells (median 0.100, IQR 0.068–0.390) compared with non-response group (median 0.044, IQR 0.024–0.070) (Table 4-7). In contrast, no significant differences were observed in exhaustion marker expression on $CD8^+$ T-cells between response group and non-response group.

Table 4-7 Comparison of exhaustion marker expression on $CD4^+$ and $CD8^+$ T-cell between immunocompromised T-cell non-response group and response group at V3+28 days

				$CD4^+CD28^+$				
Exhaustion markers				T-cell non-response group		T-cell response group		
CTLA4	LAG3	PD-1	TIGIT	Median	IQR	Median	IQR	p-value*
-	+	-	+	0.025	0.006-0.092	0.052	0.019-0.175	0.387
-	+	-	-	0.240	0.120-0.405	0.190	0.135-0.390	1.000
-	+	+	+	0.050	0.018-0.115	0.210	0.045-0.585	0.103
-	+	+	-	0.032	0.020-0.125	0.066	0.028-0.520	0.425
-	-	-	+	4.710	4.015-6.195	11.800	6.035-14.600	0.123
-	-	+	+	0.880	0.530-1.445	3.430	1.855-11.850	0.070
-	-	+	-	1.800	1.135-1.950	2.100	1.570-4.620	0.246
+	+	-	+	0.000	0.000-0.013	0.005	0.000-0.010	1.000
+	+	-	-	0.015	0.000-0.042	0.039	0.016-0.058	0.235
+	+	+	+	0.008	0.000-0.020	0.042	0.019-0.104	0.050
+	+	+	-	0.042	0.018-0.048	0.045	0.025-0.180	0.364
+	-	-	+	0.240	0.190-0.430	0.290	0.175-0.440	1.000
+	-	+	-	0.100	0.029-0.175	0.160	0.074-0.585	0.189
+	-	+	+	0.044	0.024-0.070	0.100	0.068-0.390	0.035
+	-	-	-	0.480	0.340-0.580	0.410	0.265-0.580	0.618

				$CD8^+CD28^+$				
Exhaustion markers				T-cell non-response group		T-cell response group		
CTLA4	LAG3	PD-1	TIGIT	Median	IQR	Median	IQR	p-value*
-	+	-	+	0.094	0.066-0.150	0.200	0.114-0.460	0.364
-	+	-	-	0.290	0.140-0.660	0.410	0.375-0.565	0.328
-	+	+	+	0.000	0.000-0.046	0.035	0.015-0.215	0.140
-	+	+	-	0.000	0.000-0.066	0.064	0.013-0.260	0.117
-	-	-	+	6.990	4.870-10.600	9.310	3.755-18.350	0.659
-	-	+	+	1.310	0.830-1.830	2.940	1.495-6.320	0.069
-	-	+	-	1.220	0.945-2.905	1.890	1.505-5.160	0.246
+	+	-	+	0.000	0.000-0.000	0.000	0.000-0.029	0.051
+	+	-	-	0.000	0.000-0.000	0.000	0.000-0.009	0.093
+	+	+	+	0.000	0.000-0.021	0.000	0.000-0.016	0.757
+	+	+	-	0.000	0.000-0.000	0.000	0.000-0.026	0.359
+	-	-	+	0.000	0.000-0.075	0.020	0.000-0.042	0.924
+	-	+	-	0.000	0.000-0.015	0.000	0.000-0.014	0.757
+	-	+	+	0.000	0.000-0.032	0.000	0.000-0.012	0.787
+	-	-	-	0.030	0.000-0.054	0.013	0.000-0.029	0.670

*p-value was calculated using Wilcoxon rank-sum test.

Class-switched B-cells ($IgM^{-}IgD^{-}$) were additionally assessed as an indicator of B-cell maturation, as these cells are frequently impaired in immunocompromised individuals. Frequencies of class-switched B-cells did not differ significantly between the T-cell non-response group (median 5.92%, IQR 3.04–14.50) and response group (median 1.54%, IQR 0.26–6.86), with a trend toward higher levels in non-response group although it did not reach statistical significance (p = 0.07) (Figure 4-15). Consistent with the observed B-cell profiles, all T-cell non-response group (100%, 7/7) demonstrated a positive antibody response one month after the third vaccine dose, whereas 54.5% (6/11) of T-cell response group mounted a detectable antibody response.

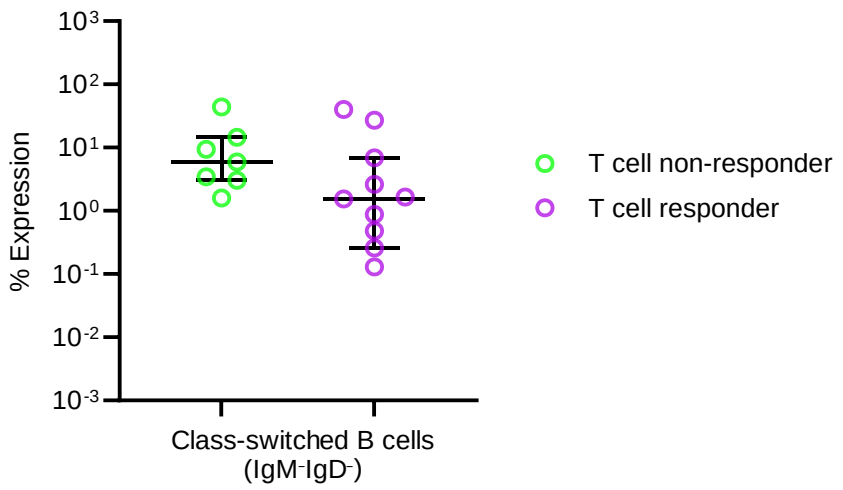

Figure 4-15 Comparison of class-switched B-cell frequencies between immunocompromised T-cell non-response group and response group at V3+28 days

Peripheral blood mononuclear cells (PBMCs) were obtained from immunocompromised individuals classified as T-cell response group (n = 11) or non-response group (n = 7) based on SARS-CoV-2 spike-specific ELISpot responses one month after the third dose of BNT162b2. Class-switched B-cells were defined as $IgM^{-}IgD^{-}$ B-cells. Data are presented as medians with interquartile ranges (IQR), with individual data points shown. The comparison between groups were analysed using the Wilcoxon rank-sum test.

4.3 Discussion

In this study, I investigated whether baseline immune competence and immunophenotyping post vaccination could explain inter-individual variability in vaccine-induced T-cell responsiveness following COVID-19 vaccination. I first explored whether blood-based immune markers could be used to predict T-cell responsiveness following vaccination.

Both serum and plasma collected prior to the second dose of vaccine were included in the analysis due to limited sample availability. Plasma retains fibrinogen and other coagulation factors, contributes to higher variability between samples as observed by the broader dispersion in the PCA plot and may affect the sensitivity for detecting certain protein signals. In contrast, the removal of clotting-associated proteins during serum preparation results in more homogeneous proteomic profiles, as shown by the tighter cluster in PCA. However, clot formation can induce platelet activation and release of proteins leading to increase of some proteins in serum. Nonetheless, overall proteomic profiles were broadly comparable between serum and plasma. Differences observed for a subset of proteins were consistent with known effects of coagulation-related platelet activation, including enrichment of signalling and chemotaxis-associated proteins in serum. Given the absence of clear separation between biomaterials at the global level, combined analysis was considered appropriate and to minimise potential confounding effect, sample type was accounted for in the prediction model.

Using pre-V2 blood samples, this study identified a small panel of circulating proteins that independently predicted vaccine-induced T-cell responsiveness in healthy individuals, indicating that baseline immune competence shapes subsequent cellular responses to vaccination. In multivariable mixed-effects modelling, higher baseline levels of eIF5A and COL9A1 were associated with increased odds of mounting a T-cell response, whereas

elevated IL1RL2, MMP-1, and SKAP2 were associated with reduced responsiveness. These associations remained significant after adjustment for age, sex, and CMV serostatus. Many of the identified proteins likely act as indirect markers of the immune environment rather than as direct regulators of T-cell activation, reflecting immune competence that shapes vaccine-induced responses.

eIF5a, which was positively associated with T-cell responses following vaccination, has been previously been identified as one of proteins that was found abundant in cytotoxic T-cells (Hukelmann et al., 2016). A study in mice showed that the hypusination of eIF5a protein, mice showed impaired T-cell differentiation and effector function, including reduced IFNγ and TNF production, resembling immunosenescence (Tan et al., 2022). Spermidine, which declines with age, can be supplemented to improve eIF5A and TFEb and restore human $CD8^+$ T-cell function (Alsaleh et al., 2020). Hence, the level of eIF5a prior to vaccination may represent the host immune competence to induce T-cell differentiation and effector T-cell responses following mRNA vaccination and supplementation to boost eIF5a level may improve vaccine responses in ageing population.

Two extracellular matrix (ECM)-related proteins, COL9A1 and MMP-1, were also associated with vaccine-induced T-cell responsiveness. COL9A1 was positively associated with T-cell responsiveness, while MMP-1 was associated with reduced responsiveness. The ECM role is known in regulating immune responses by controlling immune cell positioning and movement, while immune cells can also modify the ECM through cytokines, chemokines, and matrix-remodelling enzymes (Sutherland et al., 2023). *Col9a1*$^{-/-}$ knock-out mice had reduced myeloid cell numbers and impaired innate responses upon encounter with pathogen (Probst et al., 2018). Although the number of B cells and T cells were not impacted in *col9a1*$^{-/-}$ knock-out mice (Probst et al., 2018)., a collagen-type-1 study showed

that collagen viscoelasticity affects the T-cell population generated (Adu-Berchie et al., 2023). Slow-relaxing matrices had more activated T-cells, while fast-relaxing module had higher memory T-cells (Adu-Berchie et al., 2023). In contrast, matrix metalloproteinases play a central role in extracellular matrix degradation and remodeling (Khokha et al., 2013). MMP-1 is upregulated during classical macrophage activation, leading to increased collagenase activity (Huang et al., 2012), associated with chronic inflammation and senescence-associated secretory phenotype (Basisty et al., 2020). Hence, tissue environment pre-vaccination may determine the effectiveness of vaccine-induced response. Supportive matrix facilitates effective immune priming, whereas matrix remodeling due to chronic inflammation may impair vaccine-induced T-cell responses.

SKAP2 and IL1RL2 were associated with reduced odds of T-cell responsiveness and are involved in inflammatory and myeloid signalling. SKAP2 is required for macrophage and neutrophil migration and adhesion, by inducing actin cytoskeletal arrangement, during inflammation and metastatic of cancer cells (Alenghat et al., 2012; Bouti et al., 2024). T-cell proliferation and effector function are not impaired in SKAP2-deficient mice, unlike B-cell proliferation (Togni et al., 2005). Thus, SKAP2 does not directly control T-cell signaling, instead serves as a biomarker of myeloid dysfunction or chronic inflammation. In contrast, IL1RL2 mediates IL-36 cytokine signalling and promotes proliferation of $CD4^+$ T-cells (Vigne et al., 2012), $CD8^+$ T-cells and B-cells (Aoyagi et al., 2017; Penha et al., 2016). However, sustained activation of IL1RL2/IL-36 may reflect inflammatory stress, which potentially contribute to the pathogenesis of COVID-19 (Manzanares-Meza et al., 2022). The inflammatory environment as reflected by high level of SKAP2 and IL1RL2 prior to vaccination may therefore limit the effective induction of vaccine immunity.

To assess the translational potential of the proteomic predictors identified in this study, selected proteins were evaluated for detectability using a clinically accessible assay platform (ELISA) and for agreement with Olink-based measurements. Consistent with prior reports, proteins differed substantially in their detectability by different platforms depending on their cellular localisation and abundance (Karlsson et al., 2021). Among the candidates examined, SKAP2 emerged as a potential marker for further translational development, given its detectability by ELISA, consistent association with T-cell responsiveness, and moderate cross-platform agreement. In contrast, some predictive proteins may be better suited to high-sensitivity proteomic platforms, underscoring the need for optimisation during translation to clinically applicable assays.

Increasing age was positively associated with the odds of being a T-cell response group within a cohort of adults ≤65 years. The positive association with age appears to contrast with multiple reports linking advanced age to impaired vaccine responses (Collier et al., 2021; Costa et al., 2022; Swanson et al., 2021). However, the present analysis did not include individuals ≥65 years, in which immunosenescence is most consistently observed (Nehme et al., 2020; Pawelec, 2019; Zhang et al., 2024). Ageing is also increasingly recognised as a heterogeneous process, in which chronological age alone does not uniformly predict immune competence. A previous study has shown that older individuals may retain cytokine-producing capacity, but reduced proliferation capacity (Jo et al., 2021). Older adults may accumulate greater antigenic exposure over time compared to younger adults, contributing to a more differentiated and activated T-cell compartment, but not senescent state that is observed in elderly population.

In contrast, latent CMV infection represents a potent driver of immune remodeling. The prevalence of CMV increases with age, however younger individuals who are seropositive

also undergoing immune remodeling (Gong et al., 2025). CMV-seropositive individuals have larger memory T-cells (EM (CCR7 CD45RA^{-}), and TEMRA (CD45RA^{+}CCD7^{-}) and fewer naïve phenotype (CD45RA^{+}CCD7^{+}CD28^{+}), than seronegative individuals (Jo et al., 2021). The increase of terminally differentiated T-cells and fewer naïve T-cells limit the T-cell repertoire for de novo priming and expansion of T-cells following vaccination.

Targeted immunophenotypic analysis further revealed that vaccine-induced T-cell responsiveness in healthy individuals was associated with distinct patterns of immune regulation and differentiation, whereas non-responsiveness was characterised by features consistent with immune senescence and functional exhaustion. In healthy vaccine response group, higher frequencies of Treg cells were observed compared with non-response group. The higher Treg frequencies observed in COVID-19 vaccine response group in this study has been reported in other mRNA vaccine studies (Franco et al., 2023; La Gualana et al., 2023), suggesting a regulatory response following activation. mRNA vaccine-induced Tregs have been shown to express CCR6, suggesting a role in modulating local inflammation at antigen exposure sites (Franco et al., 2023). However, my finding appears to be in contrast with elevated regulatory populations, including both Treg and Breg cells, which were associated with poor influenza vaccine responsiveness in older population (Riese et al., 2022). This difference likely reflects transient regulatory responses that support immunity in healthy adults, compared with the chronic expansion of regulatory cells in older individuals, which is associated with immune suppression.

In contrast, T-cell non-response group exhibited increased expression of markers associated with senescence and functional exhaustion. Although immunosenescence is most commonly linked to ageing, similar immune phenotypes can arise in the presence of chronic antigen exposure or dysregulated immune signalling, which have been associated with reduced

effector function (Brenchley et al., 2003; Effros et al., 2005; Valenzuela & Effros, 2002; Wertheimer et al., 2014). Consistent with this, the loss of CD28- on CD4+ T-cell, for example has been associated with poor antibody responses after flu vaccination in individuals over 60 years old with CMV infection (Derhovanessian et al., 2013).

However, these senescent and functional exhaustion phenotypes, were not observed among T-cell non-response group in the immunocompromised cohort, indicating that the immunological characteristics of vaccine responsiveness in this population differ from those in healthy individuals. A previous study showed that differences between T-cell response group and non-response group in immunocompromised individuals are driven by the treatment they received: treatment with calcineurin inhibitors, or corticosteroids, was associated with impaired T-cell responses in immunocompromised individuals following three doses of vaccines, while B-cell directed therapy was associated with increased odds of T-cell responsiveness (Goodyear et al., 2024). Hence, regulatory, senescent and exhaustion markers do not distinguish T-cell response group and non-response group in the immunocompromised cohort, unlike in the healthy cohort.

Comparison across healthy and immunocompromised groups showed differences in effector memory $CD4^+$ T-cells, which may reflect the number of vaccine doses received by healthy and immunocompromised patients. The differences across group in naïve $CD8^+$ T cells may represent the significant difference identified earlier within the healthy individual cohort. The contraction of naïve pool may limit the capacity to generate new antigen-specific T-cell clones, contributing to non-responsiveness or reduced responses following vaccination.

Limitations

This work has several limitations. First, the dataset was derived from a modest number of healthy adults, especially the number of non-response group in the validation dataset, so validation in larger and independent cohort to confirm this finding is required. Second, this study used both stored plasma and serum samples. Future studies should also compare the use of sample types to standardise the optimal biomaterial for clinical use. Third, although the immune competence score was developed as a proof-of-concept, not all predictive proteins are currently measurable using routine clinical assays such as ELISA. This reflects the biological nature of some markers, and future work will focus on assay optimisation or identification of surrogate markers to support translation. Fourth, analyses in this chapter focused on targeted immune features most relevant to T-cell responsiveness; a more comprehensive analysis incorporating unsupervised or semi-supervised high-dimensional approaches (e.g. clustering-based phenotyping across all measured markers) could provide additional insight into vaccine responsiveness in both healthy and immunocompromised individuals. Finally, this study was restricted to adults ≤65 years of age. This allows the investigation of immune ageing in healthy adults across four decades while minimizing confounding effects associated with age, however the findings may not be generalisable to elderly populations, which will require further studies.

Conclusions

This study provides preliminary evidence that circulating proteomic profiles and demographic factors, are associated with vaccine-induced T-cell responsiveness among healthy individuals. Although no individual proteins reached statistical significance following multiple testing correction, multivariate modelling identified some potential candidate proteins associated with T-cell responses following mRNA vaccination. This finding suggests that proteomic approach may offer insight into pre-vaccination immune

features associated with vaccine responsiveness; however, validation in larger, independent cohorts is still needed to confirm these finding. Post-vaccination immunophenotyping further demonstrated the characteristics of regulated activation and differentiation of T-cell following mRNA vaccination in those with T-cell responses, whereas markers of exhaustion or immunosenescence were higher in those without T-cell responses. In contrast, the T-cell markers of vaccine responsiveness observed in healthy individuals were not observed in immunocompromised individuals, suggesting population-specific factors influencing cellular vaccine immunity.

5 Immune responses to COVID-19 vaccination in an Indonesian community cohort: effects of vaccine platform, prior SARS-CoV-2 infection, and seasonal coronavirus immunity

5.1 Introduction

The global COVID-19 vaccination programme relied on multiple platforms, including inactivated whole virus, adenoviral vector, mRNA, and protein subunit (Fiolet et al., 2022; Sadarangani et al., 2021). The different features between platforms shape the magnitude and quality of both humoral and cellular responses. Prior SARS-CoV-2 infection and pre-existing cross-reactive immunity to seasonal human coronaviruses (HCoVs) may also modulate vaccine immunogenicity through imprinting or epitope recognition.

Inactivated vaccines deliver the whole virion, potentially generating broader immune responses beyond anti-spike immunity, such as responses against nucleocapsid, membrane and envelope proteins compared with BNT162B2 vaccines (Lim et al., 2022; Mok et al., 2022). The antigen uptake from inactivated vaccine depends primarily on exogenous cell processing, leading to presentation predominantly via MHC class II pathways. This results in strong $CD4^+$ T-cell priming but comparatively limited $CD8^+$ T-cell responses (Lim et al., 2022). Viral vector and mRNA vaccines, in contrast, deliver genetic material encoding the spike protein, enabling endogenous antigen expression within host cells (Teijaro & Farber, 2021). As a result, spike is processed through both MHC class I and class II pathways, promoting robust activation of $CD8^+$ and $CD4^+$ T-cells (Sahin et al., 2021; Swanson et al., 2021). ChAdOx1 vaccination induced CD4+ T cell, CD8+ T cells and antibody in all age groups (Ramasamy et al., 2020; Swanson et al., 2021). mRNA vaccines have been shown to induce potent and sustained germinal centre responses (Lederer et al., 2022; Turner et al., 2021), likely contributes to the higher magnitude and quality of antibody responses against

spike proteins following mRNA vaccination compared with inactivated vaccine platforms (Harris et al., 2024; Lim et al., 2022; Mok et al., 2022).

Despite the large-scale administration of COVID-19 vaccines in Indonesia, the world's fourth most populous country, head-to-head immunological comparisons of primary doses of COVID-19 vaccines within this population remain limited. Most studies in Indonesia have assessed immune responses within a single vaccination regimen, without comparing different vaccine platforms (Cucunawangsih et al., 2022; Fadlyana et al., 2021; Nency et al., 2025; Santi et al., 2022; Sinto et al., 2023; Suwarti et al., 2024). One study compared vaccine responses following two doses of CoronaVac (inactivated virus) or ChAdOx1 vaccines (Ardyanto et al., 2024) and one study compared the fractional and standard booster doses of mRNA vaccine, viral vector and inactivated vaccine following AstraZeneca or CoronaVac priming (Hart et al., 2025). Both studies only measured antibody and neutralisation activity. Immunological comparisons across vaccine platforms, including cellular responses, and the influence of previous SARS-CoV-2 infection and pre-existing HCoV immunity on vaccine responses remain insufficiently characterised in this population.

In this chapter, I sought to compare humoral and cellular immune responses following homologous vaccination with inactivated, viral vector and mRNA vaccines in infection-naïve and previously infected individuals. Using longitudinal samples collected before the first dose and after the second dose of vaccine (

Table 5-1), I quantified serum IgG and IgA and profiled $CD4^+$ and $CD8^+$ T-cells producing IFN-γ, IL-2 and TNF. I also examined how previous SARS-CoV-2 infection and pre-existing HCoV immunity influenced vaccine immunogenicity using multivariable logistic regression.

I hypothesised that 1) individuals who received mRNA and viral vector vaccines would have higher levels of antibody and T-cell responses to SARS-CoV-2 spike than those receiving inactivated vaccine, but inactivated vaccine would generate immune responses to spike and non-spike proteins; 2) infection-naïve individuals would show a greater fold-increase in immune responses between the first and second vaccine doses, reflecting their lower pre-V1 immunity, whereas previously infected individuals, who have already experienced a natural antigen exposure, would show a smaller boost post-vaccination; and 3) pre-existing cross-reactive immunity to seasonal HCoVs would not affect SARS-CoV-2 vaccine responses.

5.2 Results

5.2.1 Study population

PBMCs and serum were collected from healthy participants who received their COVID-19 vaccinations at primary health care centres, Puskesmas Ciracas and Puskesmas Cakung, or at Carolus hospital in Jakarta, Indonesia (Figure 5-1). All paired samples that were available prior to the first vaccine dose (pre-V1) and a month after the second dose (post-V2) were included for this analysis. These samples were collected between November 2021 and August 2022.

Participants received two homologous doses of either the inactivated vaccine CoronaVac/Sinovac, viral vector vaccine Oxford/AstraZeneca or an mRNA vaccine

Pfizer/BioNtech or mRNA 1273/Moderna (Table 5-1). The age distribution was similar across vaccine groups, with mean ages of 45 years (range 34-56 years) in the inactivated group, 38 years (range 28-54 years) in the mRNA group and 34 years (range 27-56 years) in the viral vector group (Table 5-1). Reported prior SARS-CoV-2 infection varied by vaccine platform: 29% (10/35) in the inactivated vaccine group, 15% (4/27) in the mRNA vaccine group, and 24% (4/17) in the viral vector group.

The time since last documented SARS-CoV-2 infection differed between vaccine groups ($p<0.05$), reflecting the phased implementation of Indonesian's national vaccination programme in Indonesia. Majority of vaccines administered during the early rollout in Indonesia were inactivated or viral vector vaccine (Fadlyana et al., 2021; Hart et al., 2025). Individuals receiving mRNA vaccines had a longer interval since prior infection (median 414 days [310-452]), compared with those receiving inactivated vaccine (median 163 days [155-206]) and viral vector vaccines (median 169 days [164-228]). The interval between the first and second doses differed by vaccine platform: 28 days for the inactivated vaccine, approximately 21 days for mRNA vaccines, and 60 days for the viral vector vaccine (Figure 5-1).

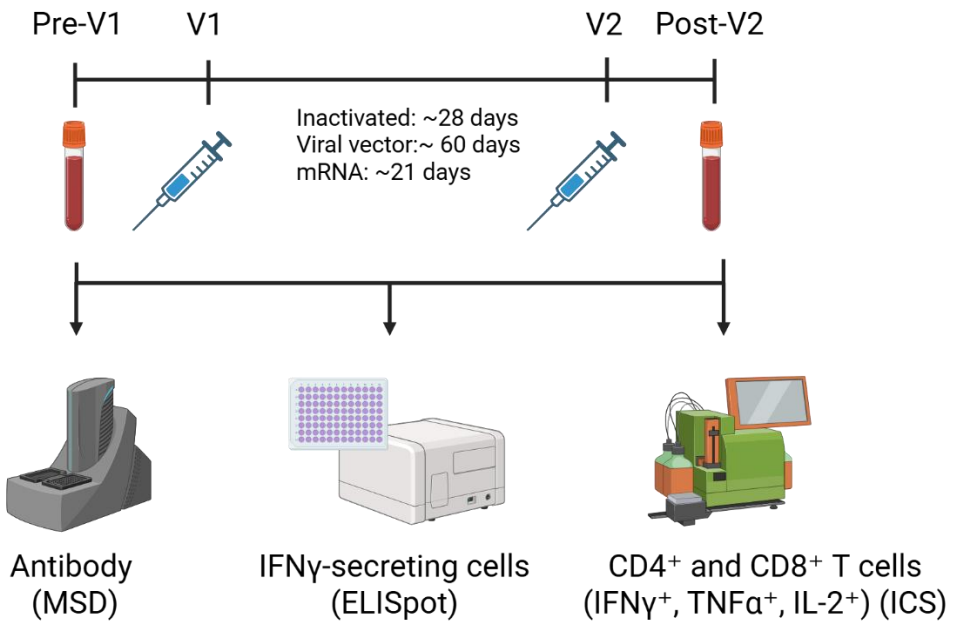

Figure 5-1 Study overview

Peripheral blood samples were collected at two timepoints: prior to the first COVID-19 vaccine dose (pre-V1) and a month after the second dose (post-V2). Participants received homologous COVID-19 vaccination with either inactivated, viral vector, or mRNA vaccines, administered according to the dosing intervals recommended by the vaccine manufacturer. All participants were healthy individuals who obtained their vaccinations at primary health care centres or hospitals in Jakarta, Indonesia. Participants were classified as naïve or previously infected based on seropositivity to SARS-CoV-2 N protein in serum before the first vaccine dose. Antibody and T-cell responses were then assessed to compare the immunogenicity of the three vaccine platforms. *Created with Biorender.com.*

Table 5-1 Demographic characteristics of vaccine groups

Characteristics	Inactivated (n=35)	mRNA (n=27)	Viral vector (n=17)	p-value*
Age, year (median, IQR)	45 (34-56)	38 (28-54)	34 (27-56)	0.5
Sex, n (%)				0.4
Male	23 (66%)	14 (52%)	12 (71%)	
Female	12 (34%)	13 (48%)	5 (29%)	
History of COVID	10 (29%)	4 (15%)	4 (24%)	0.5
Time since last infection, days (median, IQR)	163 (155-206)	414 (310-452)	169 (164-228)	0.03
Month and year of first vaccine dose	November 2021-January 2022	January 2022-Aug 2022	November 2021-April 2022	-
V1-V2 interval, days (median, IQR)	28 (28-30)	24 (21-35)	61 (58-63)	<0.001
Sample collection timepoint post-V2, days (median, IQR)	29 (28-34)	31 (28-35)	31 (30-34)	0.2

*Kruskal-Wallis rank sum test; Fisher's exact test

5.2.2 Spike-specific and nucleocapsid-specific IgG responses did not differ significantly across vaccine platforms in naïve individuals

The magnitude of circulating spike-specific and nucleocapsid-specific IgG antibodies were compared across vaccine platforms. To confirm the reported previous infection history, pre-V1 anti-N IgG levels were re-measured and used to classify participants as naïve or previously infected. At enrolment, 23% (18/79) participants reported having had COVID-19. However, the analysis of pre-V1 sera showed that 73% (58/79) of participants were seropositive to nucleocapsid. Nearly half of the nucleocapsid seronegative participants (47.6%, 10/21), those classified as naïve, had detectable spike-specific IgG (S, RBD, or NTD) at pre-V1, which may be due to the waning of anti-N IgG relative to anti-S IgG

(Lumley et al., 2021). Individuals who were seropositive for nucleocapsid were classified as previously infected, and all of these participants were also seropositive for spike (Supplementary Figure 8).

Among infection-naïve individuals, anti-S IgG levels after two doses of homologous vaccines were broadly similar across platforms (Figure 5-2). Median anti-S IgG levels were 4.7 AU/mL (IQR 4.1–5.3) in the inactivated group, 5.4 AU/mL (IQR 5.2–5.6) in the mRNA group, and 5.4 AU/mL (IQR 5.2–5.9) in the viral vector group ($p \geq 0.05$). The inactivated vaccine group showed a broader distribution of responses, with 50% of individuals (5/10) exhibiting anti-spike IgG concentrations within the lower quantile (<4.7 AU/mL) of naïve seropositive individuals (Figure 5-3). In contrast, responses in the viral vector and mRNA groups were more tightly clustered around higher median values.

The inactivated vaccine induced a 1.71-fold increase in anti-S IgG ($p<0.002$), a 1.50-fold increase in anti-RBD IgG ($p<0.02$) and a 3.08-fold increase in anti-NTD IgG in naïve individuals post-V2 compared with pre-V1 (Figure 5-3). The mRNA vaccine induced a 2.81-fold increase in anti-S IgG ($p<0.05$), a 1.74-fold increase in anti-RBD IgG ($p<0.05$) and a 3.63-fold increase in anti-NTD IgG. Changes in IgG to spike proteins after two doses of viral vector did not reach statistical significance (anti-S 1.63-fold, $p \geq 0.05$, anti-RBD 1.68-fold, $p \geq 0.05$, anti-NTD 2.34-fold $p \geq 0.05$).

Post-V2, 8/10 (80%) individuals receiving the inactivated vaccine were seropositive for anti-N IgG (Figure 5-3). Some participants who received spike-only vaccines also were seropositive for IgG to nucleocapsid: 17% (1/6) in the mRNA group and 3/5 (60%) in the viral vector group. Pairwise Fisher's exact tests indicated higher proportion of

seroconversion in inactivated group compared with the mRNA group ($p<0.05$), while no significant differences were observed between the other platforms.

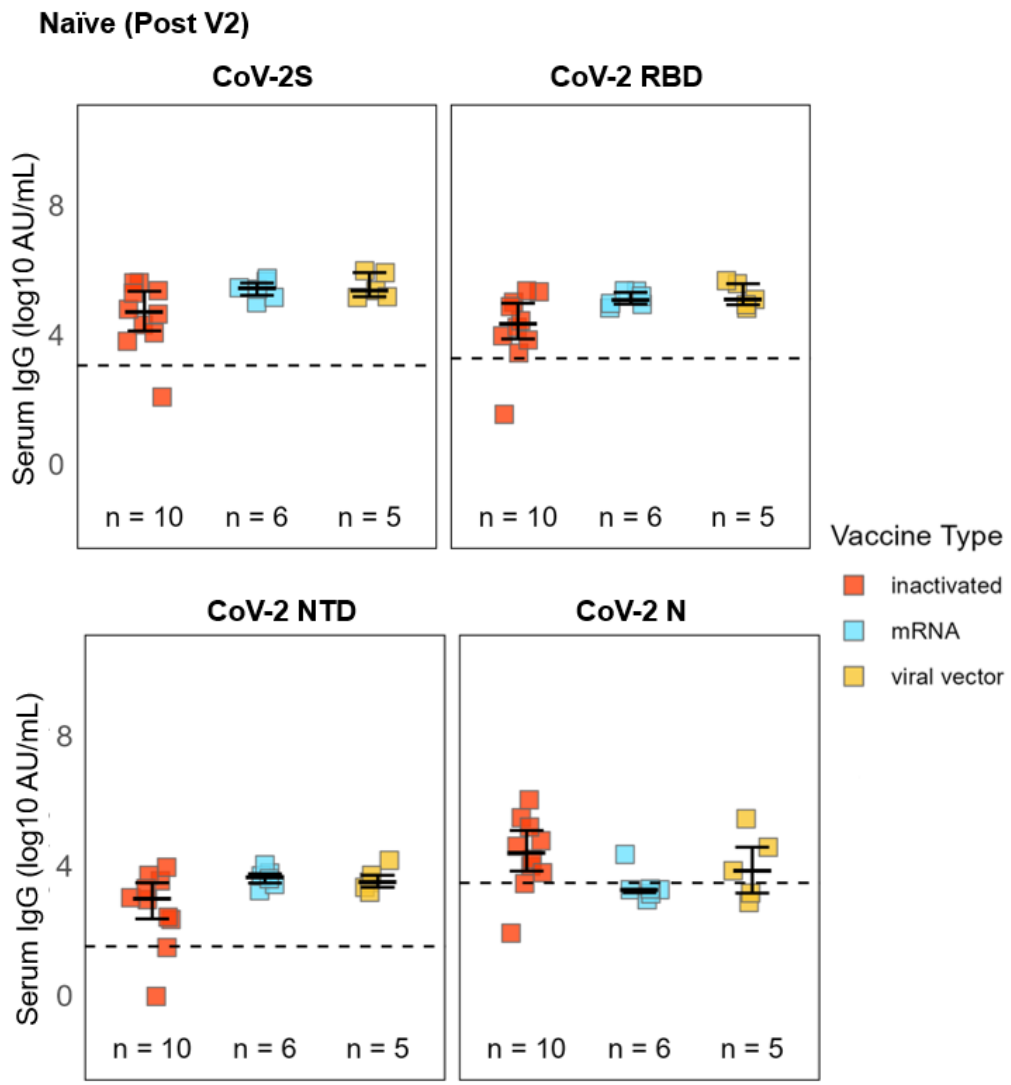

Figure 5-2 Comparison of SARS-CoV-2 specific IgG responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in naïve individuals

IgG responses to SARS-CoV-2 spike, RBD, NTD and nucleocapsid proteins post-V2 in naïve individuals receiving inactivated, mRNA, or viral vector vaccines. Median and interquartile range (IQR) with individual data points are shown. Dashed lines denote assay positive cut-offs. Group differences were assessed using the Kruskal–Wallis test with post hoc Dunn's tests (Bonferroni correction).

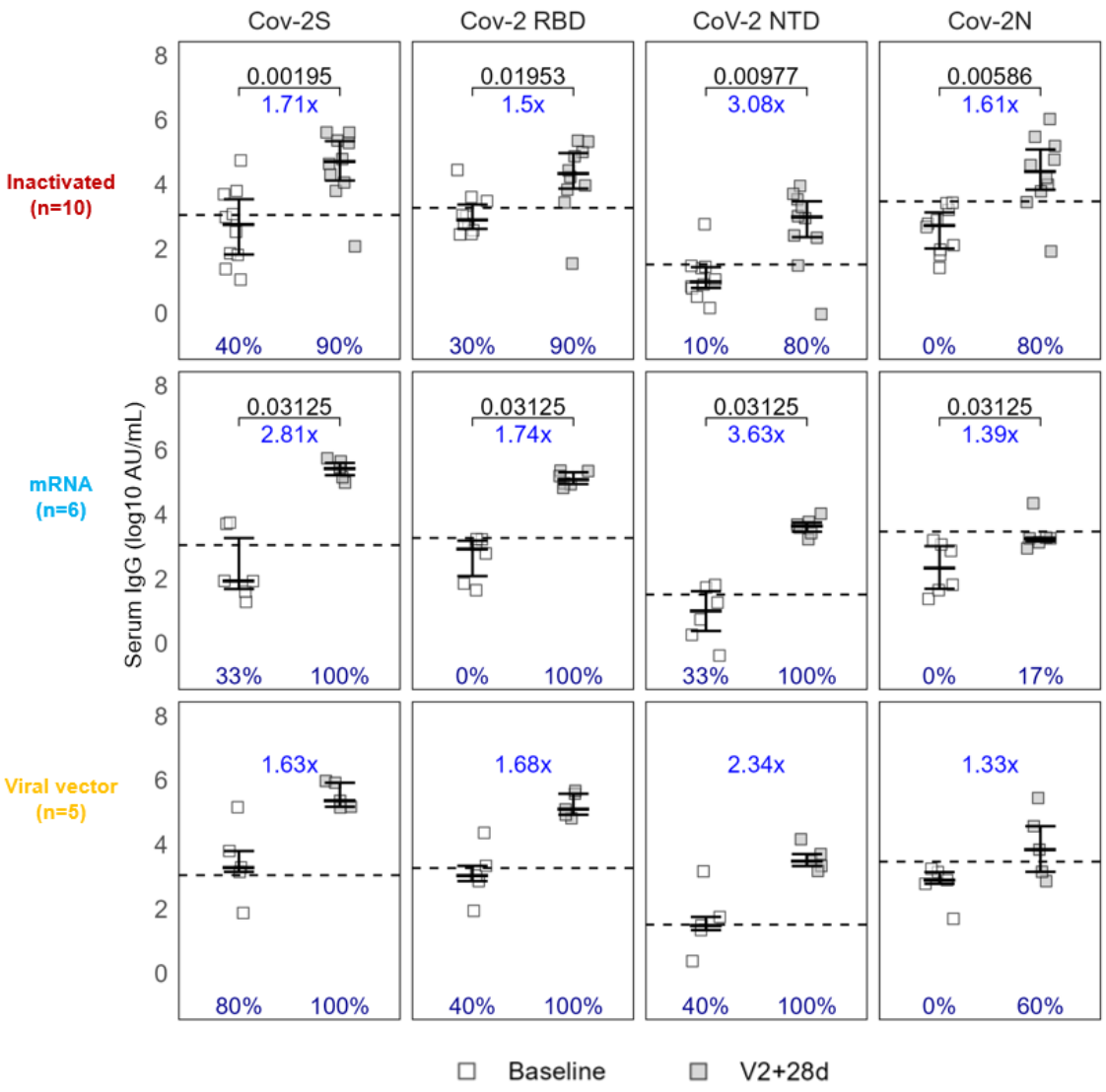

Figure 5-3 SARS-CoV-2 specific IgG responses before vaccination and one month after the second vaccine dose in naïve individuals

IgG levels to SARS-CoV-2 spike, RBD, NTD and nucleocapsid proteins pre-V1 and post-V2 in naïve individuals. Median and interquartile range (IQR) with individual data points are shown. Seropositivity proportions and fold changes are indicated. Dashed lines denote assay positive cut-offs. Paired pre- and post-vaccination comparisons were conducted using the Wilcoxon signed-rank test ($p<0.05$).

5.2.3 mRNA vaccines induced greater anti-S, anti-RBD and anti-NTD IgGs than inactivated or viral vector vaccines in previously SARS-CoV-2 infected individuals, whereas anti-N IgG responses did not differ significantly across vaccine platforms

Among previously SARS-CoV-2 infected participants, IgG responses differed significantly between vaccine platforms for anti-S (Kruskal-Wallis test χ^2=28.07, df=2, p<0.001), RBD (χ^2=28.5, df=2, p<0.001), and NTD (χ^2=27.68, df=2, p<0.001) (Figure5-4), with large effect sizes (ε^2=0.47-0.48). Post-hoc Dunn's tests with Bonferroni correction confirmed that mRNA vaccine recipients had significantly higher IgG levels to spike, RBD, and NTD than both the inactivated and viral vector groups (p<0.001). Median IgG concentrations in the mRNA group were notably higher for anti-S: 5.8 AU/mL [IQR 5.6-6.0], anti-RBD: 5.5 AU/mL [IQR 5.3-5.7], and anti-NTD: 4.0 AU/mL (IQR 3.7-4.3), compared with the viral vector group (anti-S: 5.2 [5.0-5.3], anti-RBD: 4.9 [4.6-5.0], anti-NTD: 3.3 [3.2-3.4]) and the inactivated group (anti-S: 4.4 [4.1-4.6], anti-RBD: 4.1 [3.8-5.0], anti-NTD: 2.7 [2.2-3.4]) (Figure 5-4).

A paired analysis pre-V1 and post-V2 vaccination showed that the mRNA vaccine resulted in a 1.27-fold increase of anti-S IgG (p<0.001), a 1.31-fold increase of anti-RBD (p<0.001) and a 1.58-fold increase of anti-NTD IgG (p<0.001) (Figure 5-5), In contrast, there was no significant change of IgG levels to CoV-2 spike, RBD and NTD following two doses of inactivated or viral vector vaccine. Anti-N IgG levels did not significantly change after two doses of inactivated vaccine in previously infected individuals (1.03-fold, p≥0.05).

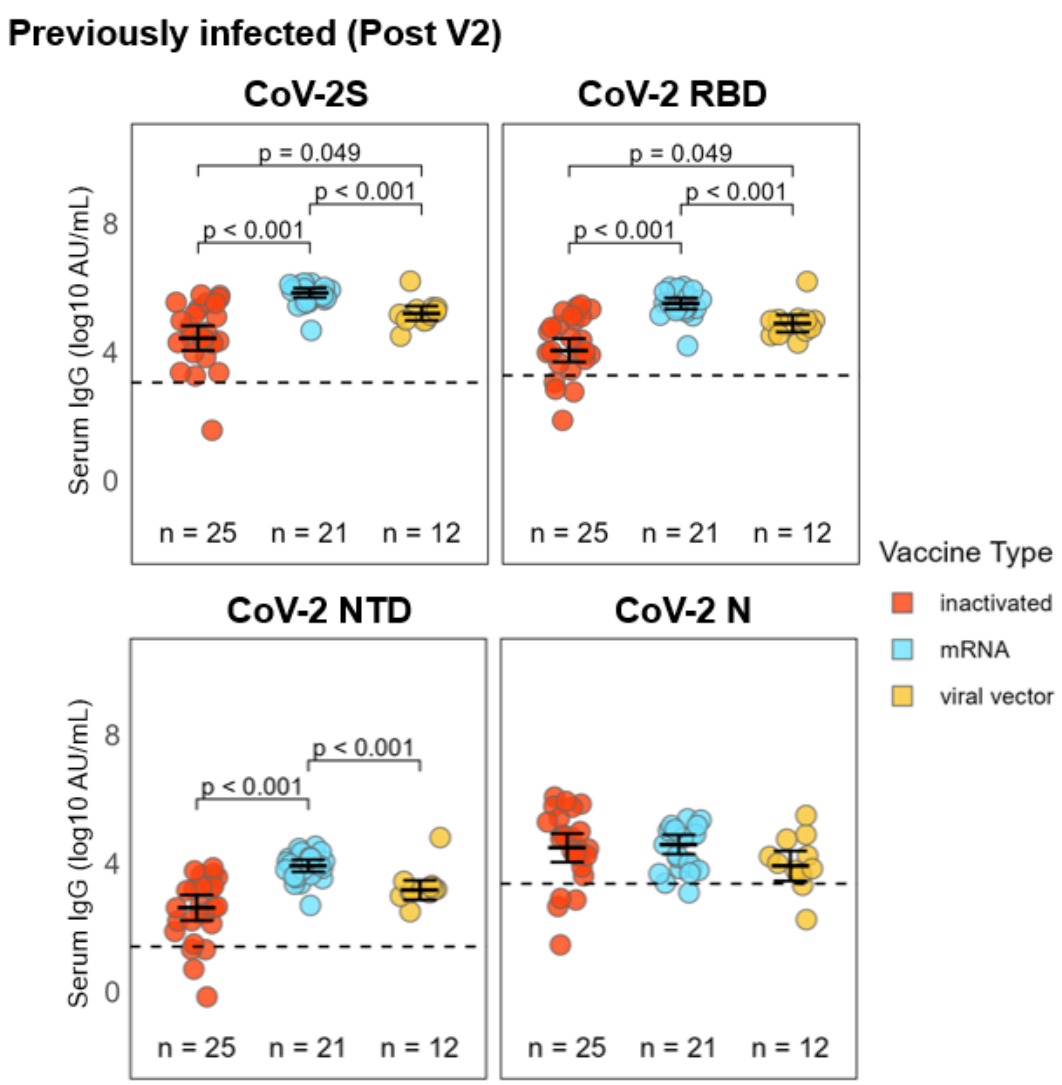

Figure 5-4 Comparison of SARS-CoV-2 specific IgG responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in previously SARS-CoV-2 infected individuals

IgG responses to SARS-CoV-2 spike, RBD, NTD and nucleocapsid proteins post-V2 in naïve individuals receiving inactivated, mRNA, or viral vector vaccines. Median and interquartile range (IQR) with individual data points are shown. Dashed lines denote assay positive cut-offs. Group differences were assessed using the Kruskal–Wallis test with post hoc Dunn's tests (Bonferroni correction).

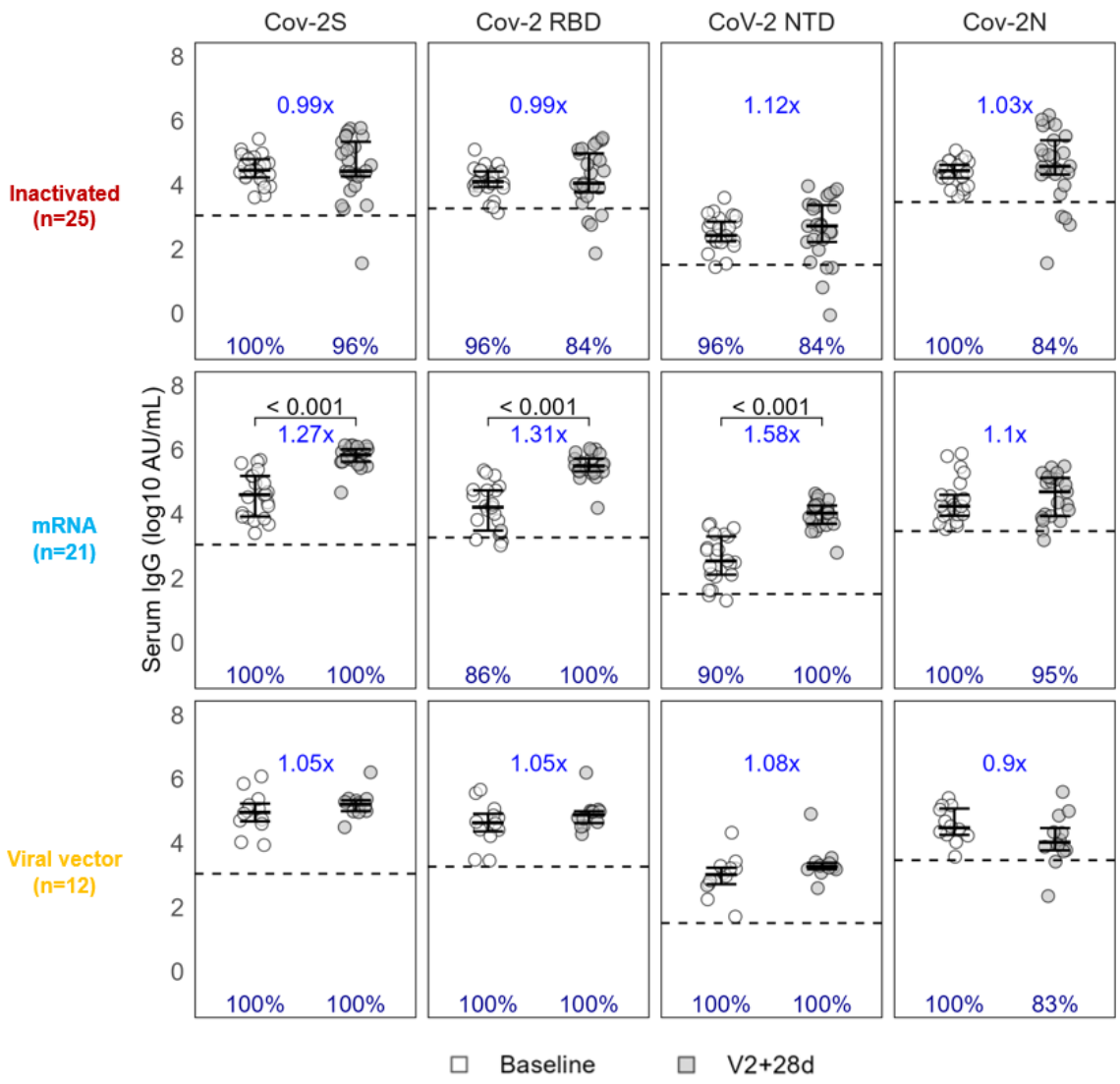

Figure 5-5 Comparison of SARS-CoV-2 specific IgG responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in previously SARS-CoV-2 infected individuals

IgG levels to SARS-CoV-2 S, RBD, NTD and N proteins pre-V1 and post-V2. Median and interquartile range (IQR) with individual data points are shown. Seropositivity proportions and fold changes are indicated. Dashed lines denote assay positive cut-offs. Paired pre- and post-vaccination comparisons were conducted using the Wilcoxon signed-rank test ($p<0.05$).

5.2.4 Spike-specific and nucleocapsid-specific IgA responses did not differ significantly across vaccine platforms in naïve individuals

Serum IgA has been shown to provide potent neutralising protection against SARS-CoV-2 infection (Sterlin et al., 2021) and in combination with IgG, serves as correlate of protection from symptomatic disease (Hertz et al., 2023). I measured serum IgA levels pre-V1 and post-V2 of COVID-19 vaccines and compared responses across vaccine platforms.

Among naïve participants, there were no differences in anti-S, anti-RBD and anti-NTD IgA levels between the three vaccine groups (Kruskal-Wallis $p \geq 0.05$) (Figure 5-6). Median anti-S IgA levels were 3.9 AU/mL [IQR 3.8–4.4] in the inactivated group, 4.4 AU/mL [IQR 4.1–4.5] in the mRNA group, 4.6 AU/mL [IQR 4.5–4.9] in the viral vector group.

Analysis of sera pre-V1 and post-V2 vaccination showed that most naïve participants developed IgA responses to S, NTD, and RBD following vaccination (Figure 5-7). A high proportion of participants in the inactivated (7/10, 70%) and viral vector (4/5, 80%) groups were positive for IgA to spike at pre-V1 (Figure 5-7, Supplementary Figure 9). After the second dose, spike-specific IgA levels did not increase significantly in the viral vector group, increased modestly in the inactivated vaccine group (1.15-fold, $p<0.05$), and showed the greatest increase in the mRNA group (1.83-fold, $p<0.05$). The inactivated vaccine group also showed a further increase in anti-N IgA, 1.15-fold ($p<0.01$), whereas no increase was detected in the mRNA (1.09-fold, $p \geq 0.05$) or viral vector (1.08-fold, $p \geq 0.05$) groups.

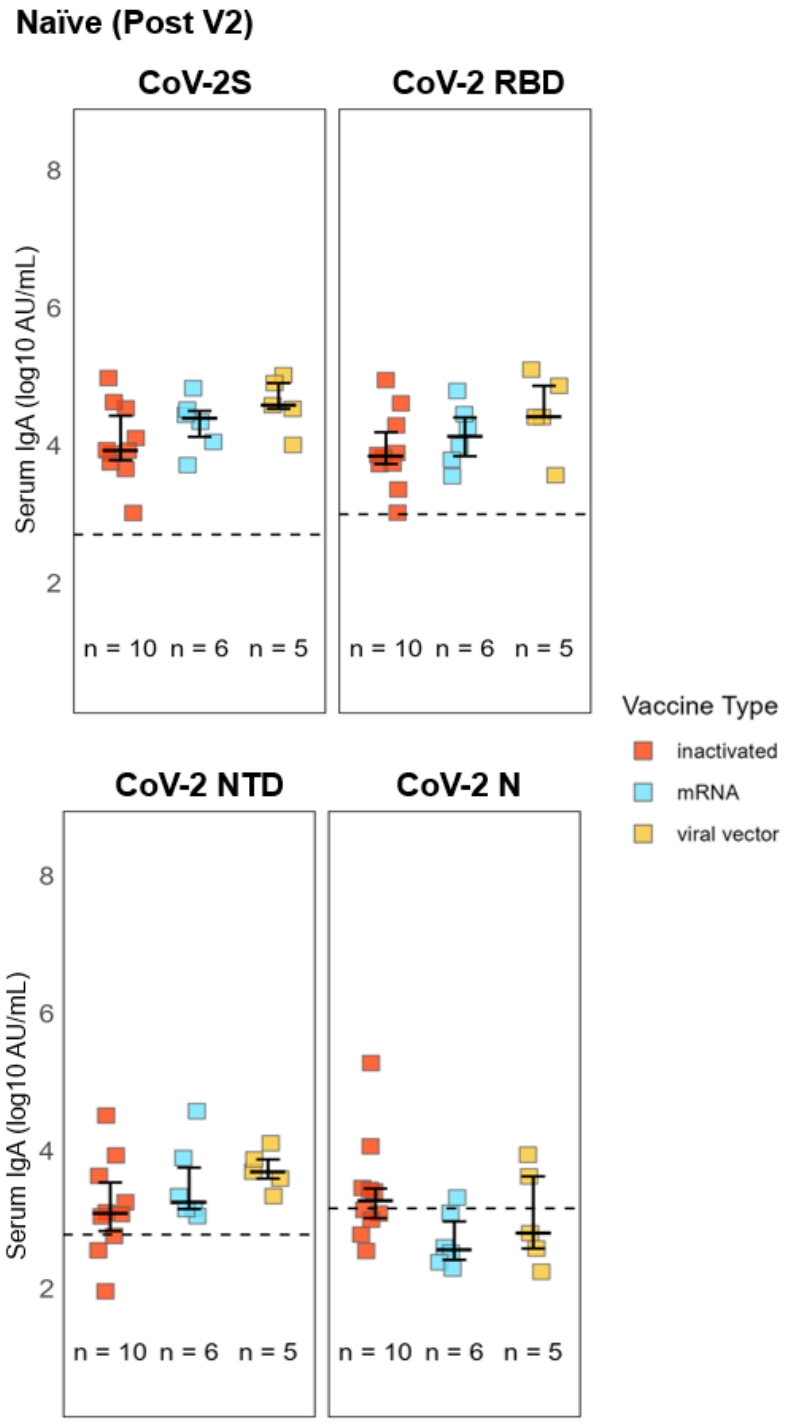

Figure 5-6 Comparison of SARS-CoV-2 specific IgA responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in naïve individuals

IgA responses to SARS-CoV-2 spike, RBD, NTD and nucleocapsid proteins post-V2 in naïve individuals receiving inactivated, mRNA, or viral vector vaccines. Median and interquartile range (IQR) with individual data points are shown. Dashed lines denote assay positive cut-offs. Group differences were assessed using the Kruskal-Wallis test with post hoc Dunn's tests (Bonferroni correction).

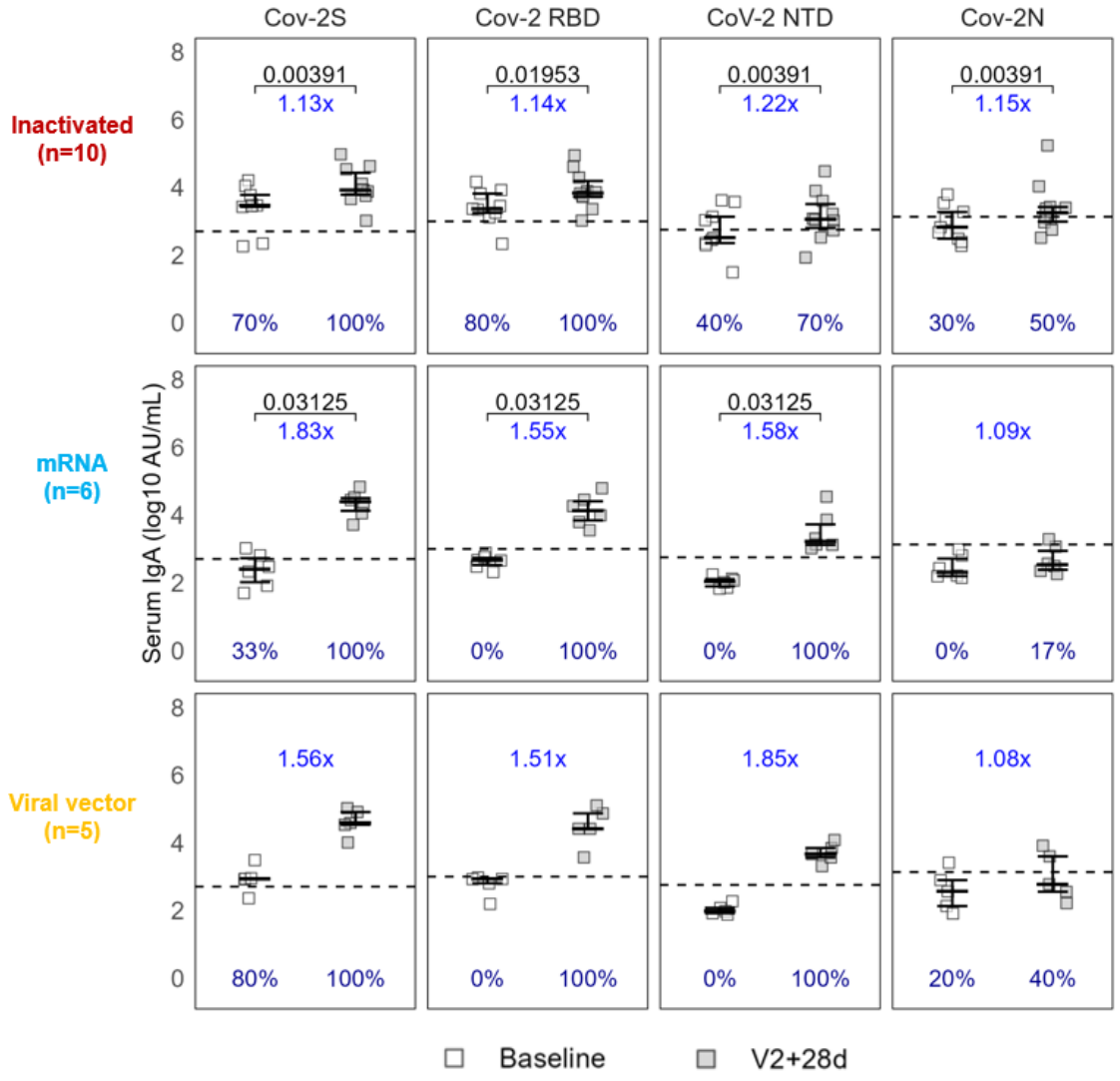

Figure 5-7 Comparison of SARS-CoV-2 specific IgA responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in naïve individuals

IgA levels to SARS-CoV-2 spike, RBD, NTD and nucleocapsid pre-V1 and post-V2. Median and interquartile range (IQR) with individual data points are shown. Positivity proportions and fold changes are indicated. Dashed lines denote assay positive cut-offs. Paired pre- and post-vaccination comparisons were conducted using the Wilcoxon signed-rank test ($p<0.05$).

5.2.5 mRNA vaccines induced greater anti-RBD IgA than inactivated or viral vector vaccines in previously SARS-CoV-2 infected individuals, whereas anti-N IgA responses did not differ significantly across vaccine platforms

In previously infected individuals, serum IgA levels differed by vaccine type: IgA to S (χ^2=9.62, df=2, p<0.01), RBD (χ^2=14.66, df=2, p<0.001), and NTD (χ^2=11.01, df=2, p<0.01) (Figure 5-8). The mRNA group showed higher serum IgA to CoV-2 S (median 4.6, IQR=4.4-4.9), RBD (median = 4.6, IQR=4.3–4.9) and NTD (median = 3.7, IQR=3.5-4.2) compared with the inactivated vaccine group (anti-S: 4.1 [IQR 3.5-4.5], anti-RBD: 4.0 [IQR 3.3-4.2], anti-NTD: 3.2 [IQR 2.7-3.7]). Serum IgA levels to SARS-CoV-2 S (anti-S: 4.3 [IQR 4.1-4.4] and NTD (3.3 [IQR 3.0–3.7]) did not differ between the mRNA and viral vector vaccine groups, but anti-RBD IgG levels were higher in the mRNA group than the viral vector group (median=4.1 [IQR 3.8-4.3]).

All three vaccines induced IgA to SARS-CoV-2 S by 1.3x (p<0.005) post-V2 (Figure 5-9). This observation differs from that of IgG, where only mRNA vaccine further boosted the antibody level in previously infected individuals (Figure 5-9). Individuals receiving inactivated vaccines also had a small increase (1.08x, p<0.001) of IgA to SARS-CoV-2 N, which was not observed in the mRNA (1.06-fold, p≥0.05) and viral vector vaccine (0.99-fold, p≥0.05) groups (Figure 5-9).

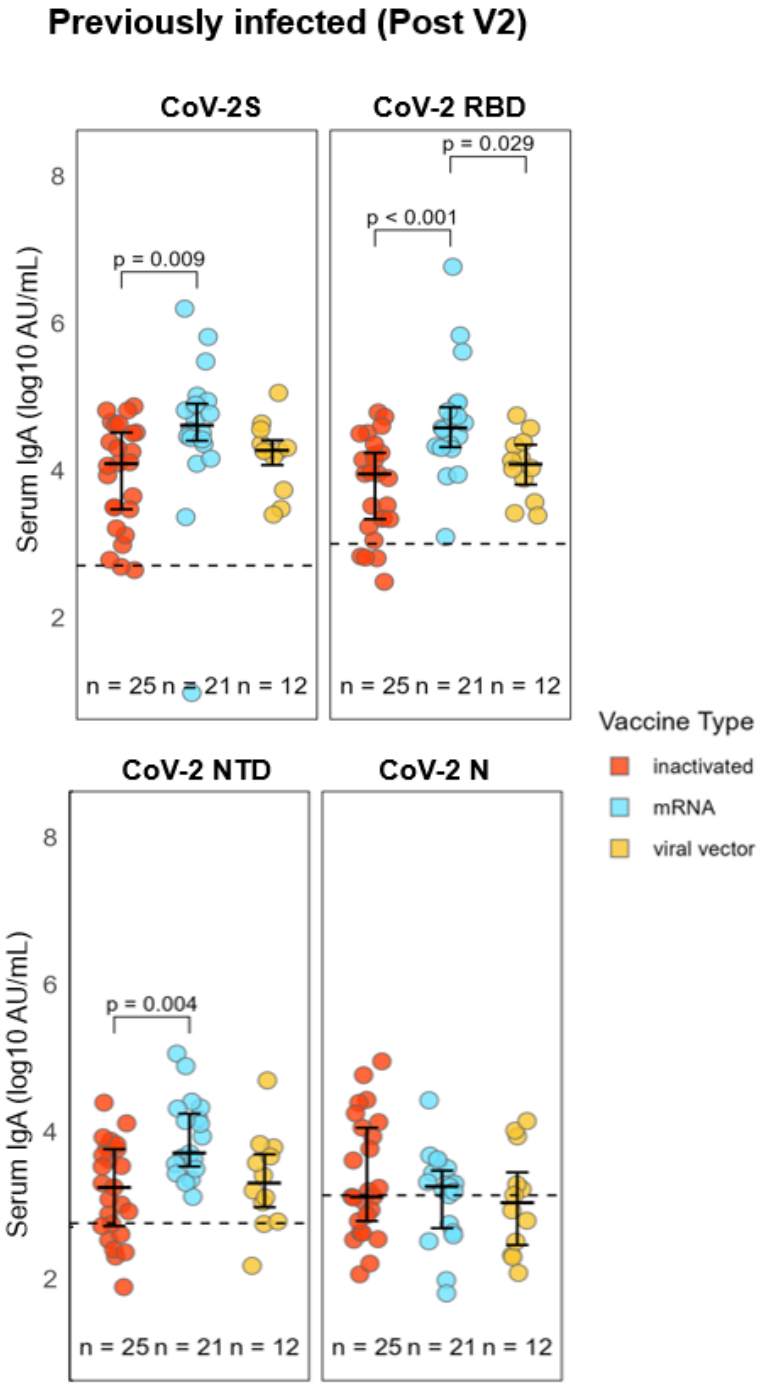

Figure 5-8 Comparison of SARS-CoV-2 specific IgA responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in previously SARS-CoV-2 infected individuals

IgA responses to SARS-CoV-2 spike, RBD, NTD and nucleocapsid proteins post-V2 in previously infected individuals receiving inactivated, mRNA, or viral vector vaccines. Median and interquartile range (IQR) with individual data points are shown. Dashed lines denote assay positive cut-offs. Group differences were assessed using the Kruskal-Wallis test with post hoc Dunn's tests (Bonferroni correction).

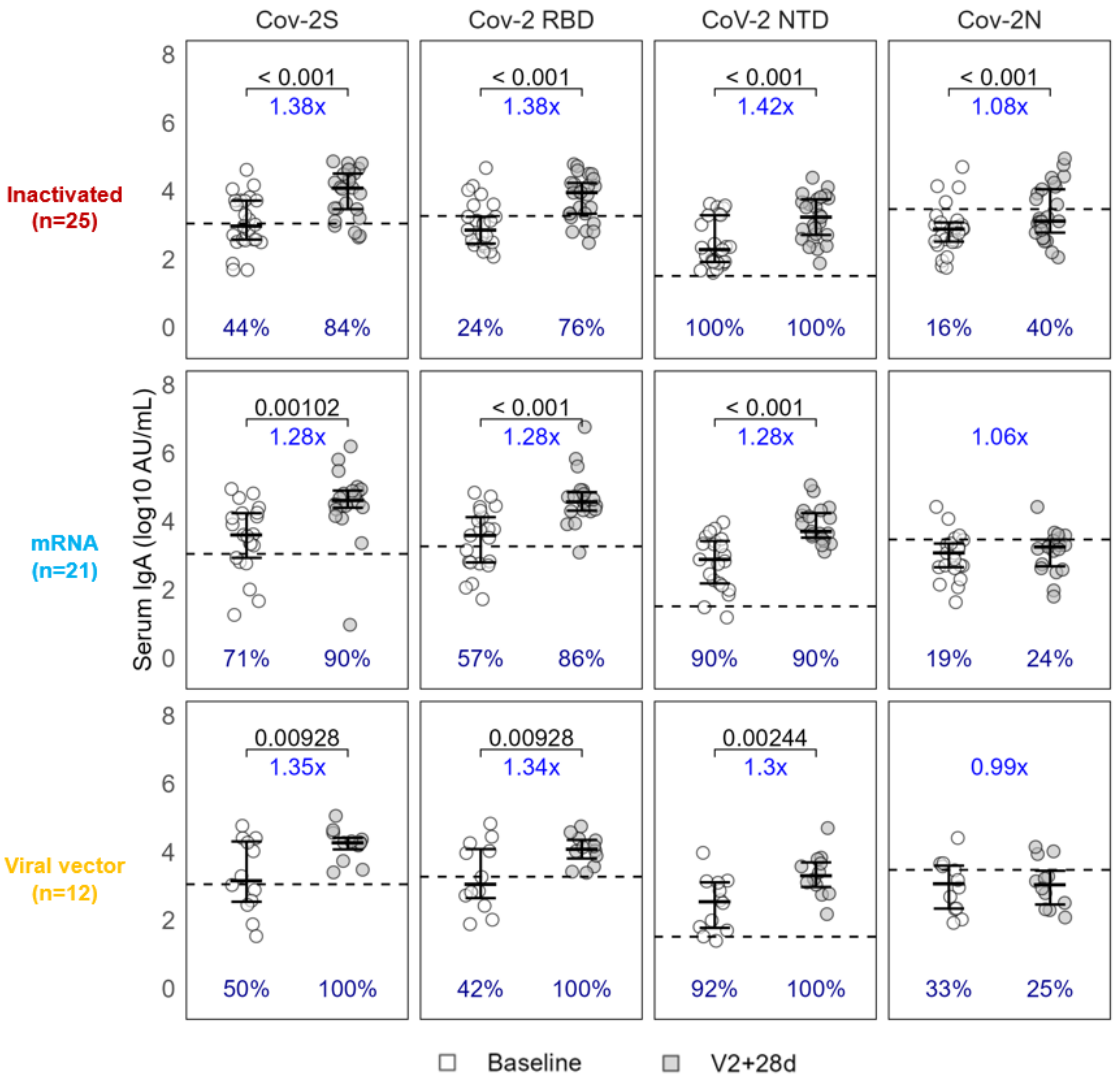

Figure 5-9 Comparison of SARS-CoV-2 specific IgA responses after two doses of either inactivated, mRNA or viral vector COVID-19 vaccines in previously SARS-CoV-2 infected individuals

IgA levels to SARS-CoV-2 spike, RBD, NTD and nucleocapsid proteins pre-V1 and post-V2. Median and interquartile range (IQR) with individual data points are shown. Positivity proportions and fold changes are indicated. Dashed lines denote assay positive cut-offs. Paired pre- and post-vaccination comparisons were conducted using the Wilcoxon signed-rank test ($p<0.05$).

5.2.6 Logistic regression analysis of factors associated with anti-S IgG increasing post-V2

To assess whether prior SARS-CoV-2 infection and pre-existing antibodies to HCoVs were associated with the likelihood of increasing IgG to SARS-CoV-2 S after vaccination, I performed univariable and multivariable logistic regression analyses (n=79). Univariable analysis showed that the odds of an anti-S IgG increasing post-V2 were higher in individuals receiving an mRNA vaccine or viral vector (OR = 3.12, 95% CI: 1.11–9.45, p = 0.035) compared with those receiving an inactivated vaccine (Table 5-2). Univariable analysis also showed that higher pre-V1 IgG levels to spike, RBD, NTD, N and CoV-1 S were associated with reduced odds of a further IgG increase following vaccination. Pre-V1 anti-HCoV S IgG or IgA levels did not significantly influence the odds of an IgG increase ($p \geq 0.05$).

Predictors identified in univariable analysis with $p<0.25$ were then included in the multivariable model (Bursac et al., 2008). These variables included vaccine type, age, pre-V1 anti-S IgG, anti-NL63 S IgG and anti-229 S IgA (Table 5-3). Pre-V1 anti-N IgG was excluded because it is strongly correlated with pre-V1 anti-S IgG (Spearman's r=0.87, $p<0.001$). The multivariable analysis showed that individuals receiving the inactivated vaccine had 96.4% lower odds of an anti-spike IgG increase compared with those receiving mRNA or viral vector vaccines (OR = 0.036, 95% CI: 0.003-0.214, p = 0.0014). Each 10-year increase in age was associated with increase in odds (OR = 1.73, 95% CI: 1.05-3.14, p = 0.045). Increase in pre-V1 anti-S IgG was significantly associated with lower odds of an IgG increase (OR = 0.04, 95% CI: 0.005–0.196, $p < 0.001$). In contrast, each $\log_{10}$ increase in pre-V1 anti-NL63 S IgG was associated with higher odds of an IgG increase (OR = 15.3, 95% CI:2.14-118.13, p = 0.011), noting the wide confidence interval for pre-V1 anti-NL63 S IgG, suggesting uncertainty of the effect size. Pre-V1 anti-229E S IgA was also associated

with higher odds of an IgG increase, but did not reach statistical significance (OR=2.73, 95% CI: 0.40-24.51).

Table 5-2 Univariable analysis of factors associated with increased IgG response to SARS-CoV-2 spike protein following two doses of homologous COVID-19 vaccine (n=79)

Predictors		Odds ratio (95% CI)	p-value
Vaccine type (ref.=inactivated)	mRNA/viral vector	3.12 (1.11–9.45)	0.035
Pre-V1 IgG level (per log10 AU/ml increase)	CoV-2 S	0.21 (0.07–0.49)	0.001
	CoV-2 RBD	0.19 (0.06–0.44)	0.001
	CoV-2 NTD	0.21 (0.07–0.47)	0.001
	CoV-2 N	0.25 (0.09–0.54)	0.002
	CoV-1 S	0.18 (0.06–0.46)	0.001
	NL63 S	2.20 (0.63–8.28)	0.226
	HKU1 S	0.49 (0.10–2.32)	0.378
	229E S	1.37 (0.29–6.60)	0.692
	OC43 S	1.14 (0.23–6.02)	0.874
Pre-V1 IgA level (per log10 AU/ml increase)	CoV-2 S	0.99 (0.55-1.78)	0.980
	CoV-2 RBD	1.14 (0.56-2.38)	0.723
	CoV-2 NTD	0.90 (0.47-1.74)	0.741
	CoV-2 N	0.84 (0.39-1.84)	0.661
	CoV-1 S	1.12 (0.54-2.37)	0.766
	NL63 S	1.02 (0.34-3.10)	0.965
	HKU1 S	0.79 (0.20-3.07)	0.730
	229E S	2.81 (0.90-9.81)	0.087
	OC43 S	0.84 (0.24-2.83)	0.784
Sex (ref.=Female)	Male	1.48 (0.52–4.16)	0.455
Age (per 10-year increase)		1.36 (0.977-1.95)	0.079
Body Mass Index (ref.=Normal)	Under/Overweight	0.91 (0.31–2.53)	0.853

Table 5-3 Multivariable analysis of factors associated with increased IgG response to SARS-CoV-2 spike protein following two doses of homologous COVID-19 vaccine

Predictors		Odds ratio (95% CI)	p-value
Vaccine type (ref.=mRNA/viral vector)	inactivated	0.036 (0.003–0.214)	0.0014
Pre-V1 IgG level (per log10 AU/ml increase)	CoV-2 S	0.04 (0.005–0.196)	<0.001
	NL63 S	15.3 (2.14–118.13)	0.011
Pre-V1 IgA level (per log10 AU/ml increase)	229E S	2.73 (0.40-24.51)	0.327
Age (per 10-year increase)		1.73 (1.05–3.14)	0.045

5.2.6.1 ACE2-binding inhibition by NL63 S-specific antibodies at pre-V1 did not correlate with IgG against SARS-CoV-2 S post-V2

As the multivariable logistic regression indicated that pre-V1 anti-NL63 S IgG was a positive predictor of SARS-CoV-2 spike IgG increase, and given that both NL63 and SARS-CoV-2 use ACE2 for cell entry (Hoffmann et al., 2020), I next investigated whether pre-existing NL63 antibodies, specifically their ACE2-inhibitory activity, were associated with post-vaccination antibody responses, as reflected by anti-SARS-CoV-2 S IgG levels or their neutralising activity.

My results showed that pre-V1 ACE2 inhibition by NL63 S-specific antibodies did not correlate with either post-vaccination SARS-CoV-2 S-specific IgG levels (Spearman's r = 0.18, p = 0.27) or ACE2 inhibition mediated by SARS-CoV-2 S-specific antibodies (r = 0.19, p = 0.23) (Figure 5-10).

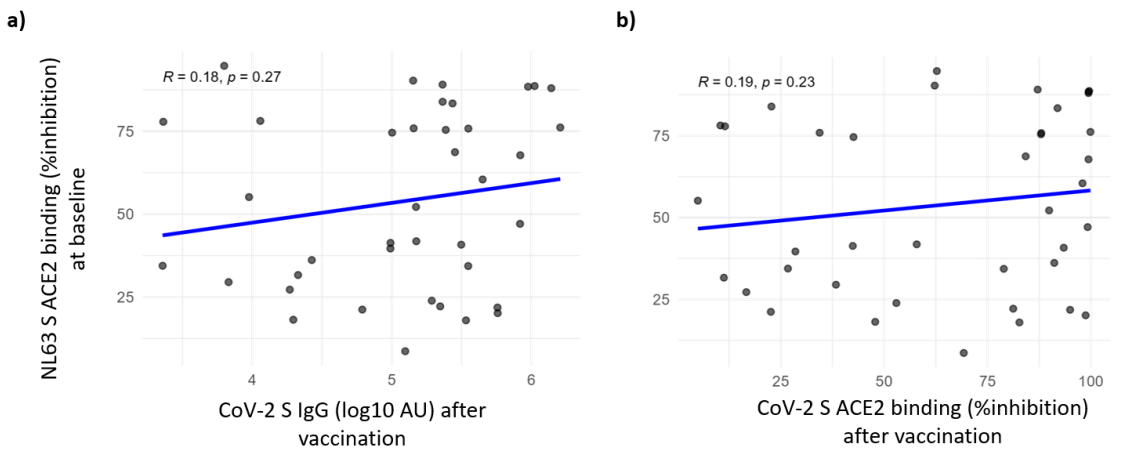

Figure 5-10 Correlation between pre-V1 NL63 S-specific ACE2 inhibition and SARS-CoV-2 antibody responses after vaccination

Correlation between ACE2-binding inhibition mediated by NL63 S-specific antibodies at pre-V1 and (a) SARS-CoV-2 S-specific IgG levels post-V2 and b) ACE2-binding inhibition mediated by SARS-CoV-2 antibodies post-V2. Correlations were assessed using Spearman's rank correlation test.

5.2.7 Comparison of seasonal coronavirus spike-specific IgGs and anti-spike T-cell responses in Indonesian and UK cohorts

IgG responses to seasonal human coronaviruses were quantified in pre-vaccination serum samples from UK and Indonesian cohorts. UK samples were collected in April 2020, whereas Indonesian samples were obtained between November 2021 and August 2022. All samples were seronegative for anti-S and anti-N IgGs prior to receiving their first dose of COVID-19 vaccine. The Indonesian cohort demonstrated significantly higher IgG titres against alphacoronaviruses and OC43 S compared with the UK cohort (Figure 5-11).

IgG levels post-vaccination could not be directly compared between the two populations because the Indonesian samples were analysed using Panel 2 MSD assay, while the UK cohort (using data previously published from PITCH study) were measured using Panel 3 MSD assay, each with its own seropositivity cut-offs.

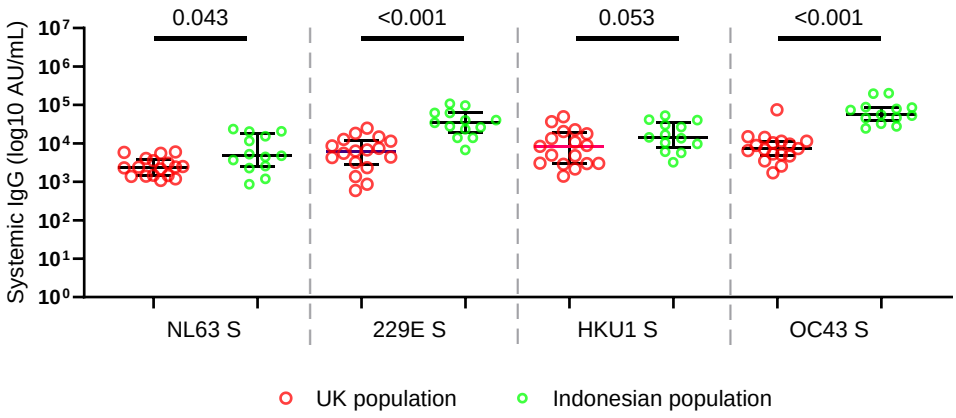

Figure 5-11 Comparison of systemic IgGs to HCoVs between UK and Indonesian populations at pre-V1

IgG responses to human seasonal coronaviruses in blood from the UK population (n=17; red dots) and the Indonesian populations (n=13; green dots) at baseline were measured using the MSD platform. Samples were seronegative for anti-SARS-CoV-2 S and anti-N IgGs. Antibody responses were measured against NL63 S, 229E S, HKU1 S, and OC43 S. Each data point represents an individual sample. Error bars represent the median with interquartile range. Comparisons between groups were performed using the Wilcoxon rank-sum test.

Given that SARS-CoV-2 vaccines rely on antigen presentation via HLA class I and II pathways, population-specific HLA backgrounds may contribute to heterogeneity in vaccine immunogenicity across geographic regions. Indonesian population showed HLA types similar to Southeast Asian populations: HLA-A*24:07, HLA-B*15:02 HLA-B*15:13, DQA1*06:01, DQB1*03:01, DRB1*12:02 and DRB1*15:02:01 (Gonzalez-Galarza et al., 2020; Tokunaga et al., 2009). UK population, largely European ancestry, more frequently carries alleles, such as HLA-A*02, HLA-B*07:02, HLA-B*08:01, DPA1*01, DPB1*04:01, DQB1*02:01, DQB1*03, DQB1*06, DRB1*04, DRB1*07:01 (Gonzalez-Galarza et al., 2020; Leen et al., 2021). Many identified class I HLA-restricted SARS-CoV-2 epitopes are associated with globally prevalent alleles that are also common in the UK population, such as HLA-A*02:01, HLA-A*24:02, HLA-B*07:02, HLA-B*08:01, whereas class II HLA-restricted SARS-CoV-2 have been reported in associations with alleles such as DRB1*07:01, DQB1*02:01, DQB1*03:01, DRB1*12:02 which are present in both UK and Indonesian populations (Grifoni et al., 2021). However, this may reflect biases in allele representation in existing studies rather than true population-specific immunodominance (Grifoni et al., 2021).

T-cell responses following two doses of mRNA vaccines were compared between UK and Indonesian population at 28 days post-vaccination (Figure 5-12). Among infection-naïve individuals, IFNγ-secreting cells to SARS-CoV-2 spike did not differ significantly between the UK (median: 157.7 SFU/10^6 PBMCs, IQR: 85.8-274.8) (n=44) and Indonesian population (median: 150 SFU/10^6 PBMCs, IQR: 122.5-760) (n=3). Among the previously infected individuals, the difference did not reach statistical significance either between the UK, median 237.5 SFU/10^6 PBMCs [IQR: 97.8-488.8] (n=24), and Indonesian population, median 130 SFU/10^6 PBMCs [IQR: 72.5-382.5] (n=7).

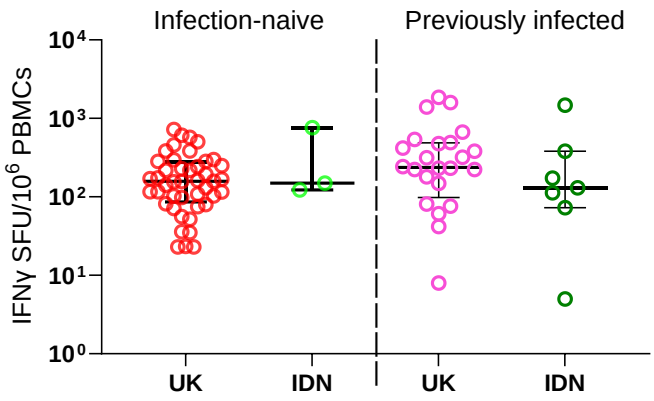

Figure 5-12 Comparison of T cell responses to SARS-CoV-2 spike between UK and Indonesian populations at V2+28 days following mRNA vaccine

The number of interferon gamma (IFN-γ)-secreting spot form unit (SFU) in response to SARS-CoV-2 spike (S) proteins after two doses of COVID-19 vaccine is shown for United Kingdom (UK) and Indonesian (IDN) population. Error bar represent the median and interquartile range (IQR), with individual data points shown. Group comparisons were performed using the Wilcoxon rank-sum test. Statistical significance is indicated if p-value<0.05.

5.2.8 No statistically significant differences in IFN-γ T-cell responses observed across vaccine platforms

To evaluate T-cell responses induced by different COVID-19 vaccine platforms, I performed IFN-γ ELISpot assays on PBMCs. Unfortunately, a substantial proportion of these samples, 70% (126/180), did not meet the quality control criteria and were excluded from data analysis (Methods Section 2.7). Exclusion criteria included high background responses (>50 SFU/10^6 PBMCs) in the negative control wells (DMSO) or insufficient responses (<200 SFU/10^6 PBMCs) in the positive control wells (ConA) (Supplementary Figure 10). This reduced the statistical power of this analysis, limiting the ability to draw conclusions from the ELISpot data or perform paired analyses.

At pre-V1 55.6% (5/9) of individuals classified as naïve, had T-cell responses to S1, 60% (3/5) had T-cell responses to N, despite being seronegative to IgG to N (Figure 5-13, baseline). After two doses of COVID-19 vaccine, a substantial proportion of mRNA vaccine recipients, in a combined analysis of naïve or previously infected, mounted T-cell responses to S1 (7/11, 64%) and S2 (9/11, 82%) (Figure 5-13, post-V2). The inactivated vaccine also induced T-cell responses to S and MN in 60% (3/5) individuals, whereas responses in the mRNA group were more limited. Overall, T-cell responses to S1, S2, or MN antigens did not differ significantly between the three vaccine platforms in naïve and previously infected individuals, but this analysis is likely to be under-powered (Figure 5-13).

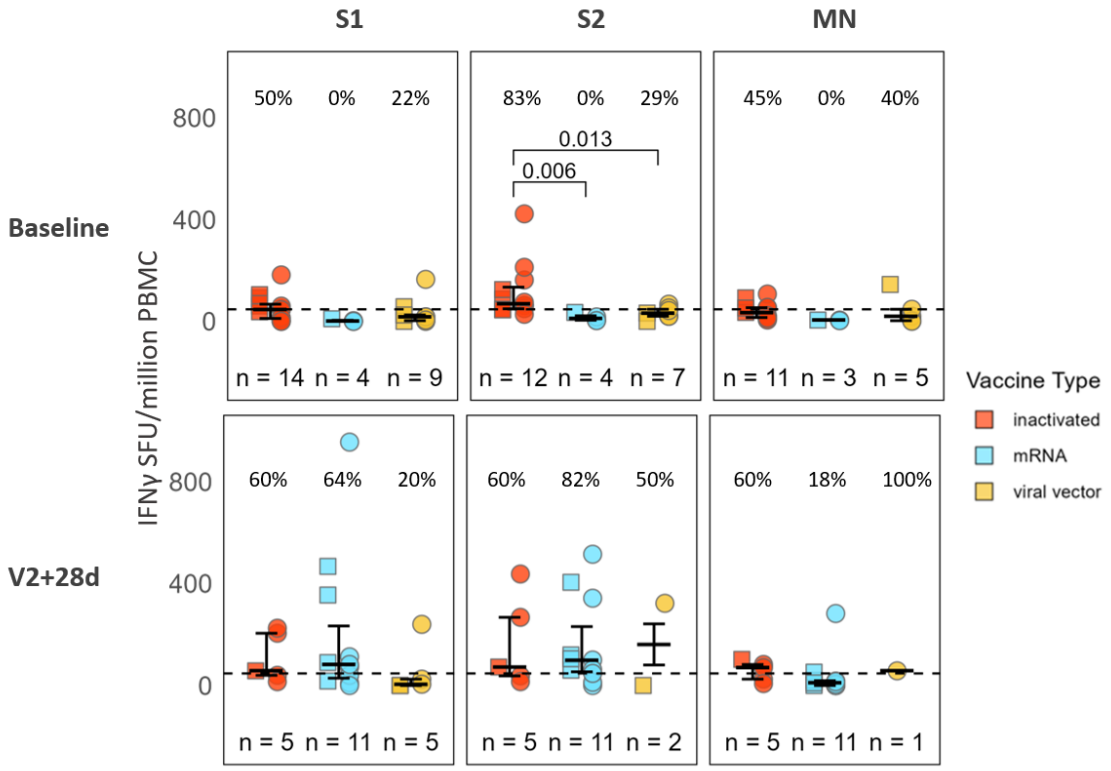

Figure 5-13 T-cells responses post-V2 of either inactivated, mRNA or viral vector COVID-19 vaccines in naïve and previously SARS-CoV-2 infected individuals

The number of interferon gamma (IFN-γ)-secreting spot form unit (SFU) in response to SARS-CoV-2 spike (S) and nucleocapsid (N) proteins after two doses of COVID-19 vaccine is shown for each vaccine groups. Error bar represent the median and interquartile range (IQR), with individual data points shown. Rectangular represents datapoint from naïve individuals and circle represents datapoint from previously infected individuals. The proportion of positive individuals is shown above the datapoints. Dashed line represents cut-off value for T-cell responses. Vaccine group comparisons were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Statistical significance is indicated if p-value<0.05.

5.2.8.1 Samples with high ELISpot background showed increased IFN-γ$^+$ NK cells and bystander CD8$^+$ T-cells activation

To determine whether elevated background responses in the ELISpot assay reflected true immune activation, I first performed intracellular cytokine staining (ICS) to identify the cellular sources of non-specific IFN-γ production. Samples with high ELISpot background exhibited significantly higher frequencies of IFN-γ$^+$ NK cells (Wilcoxon rank-sum test, $p<0.05$) (Figure 5-14). In contrast, IFN-γ expression did not differ significantly among CD4$^+$ T-cells, CD8$^+$ T-cells, double-negative (CD4$^-$CD8$^-$) T-cells, or invariant natural killer T (iNKT) cells, indicating that NK cells may be a key contributor to background IFN-γ production.

To further assess whether elevated ELISpot background was associated with T-cell activation, I performed extracellular staining (ECS) flow cytometry on a subset of five participants who had paired samples available, one timepoint with high background response and one timepoint with low background, to control for inter-individual variability. CD69 and HLA-DR were used as markers of early and late T-cell activation, respectively.

Although CD4$^+$ and CD8$^+$ T-cells appeared to have higher CD69 expression in samples with high ELISpot background, this observation was not statistically significant (Figure 5-15a). The Wilcoxon signed-rank test did not identify a consistent directional trend across the paired samples. No significant differences were observed in HLA-DR expression between samples with and without elevated T-cell responses in the control wells. Frequencies of CD8$^+$CD69$^+$ T-cells showed a moderate positive correlation with background IFN-γ spot counts in unstimulated ELISpot wells (Spearman's $r = 0.68$, $p = 0.035$) (Figure 5-15b). As CD69 upregulation and IFN-γ production are both markers of T-cell activation, the presence

of IFN-γ spots in negative wells likely reflects a degree of non-specific, bystander immune activation.

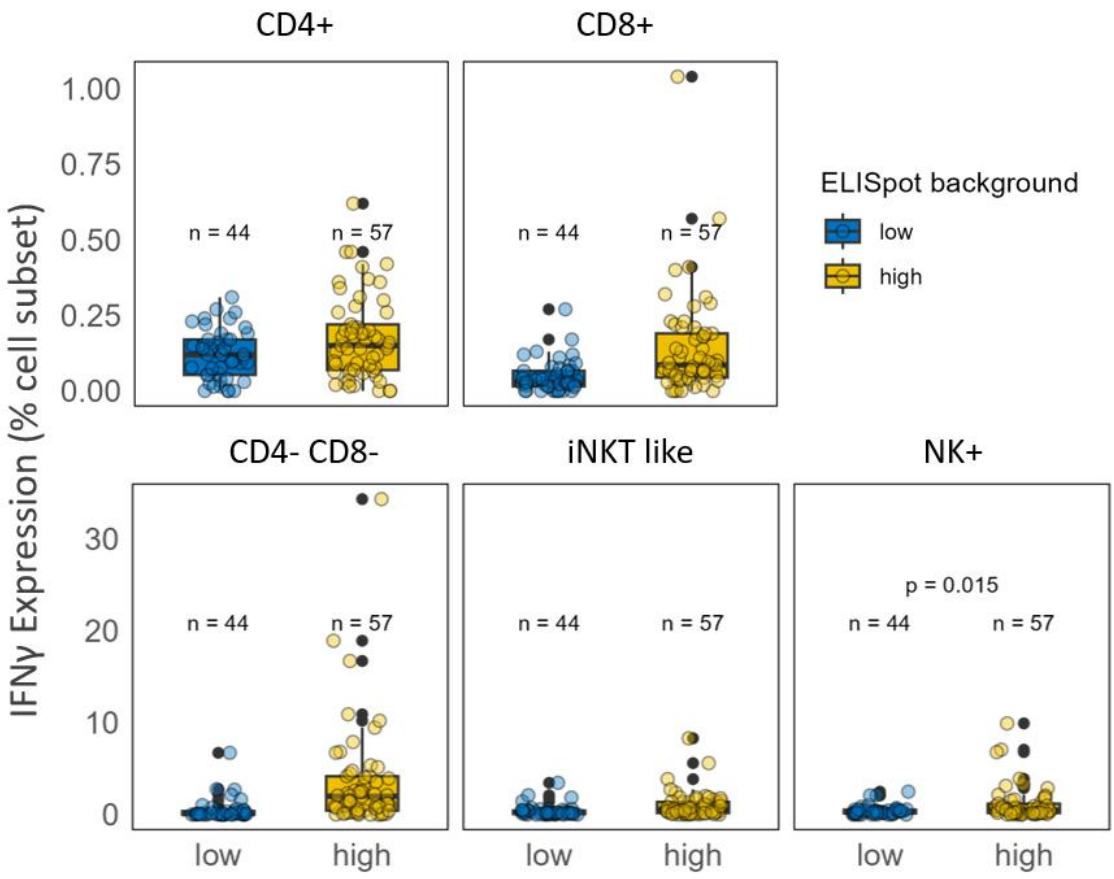

Figure 5-14 Comparison of IFN-γ expression across different cell types in samples with and without high background in ELISpot

Peripheral blood mononuclear cells (PBMCs) were stimulated with DMSO for 16 hours. The proportion of IFN-γ expressing cells among various PBMC subsets was measured using intracellular cytokine staining (ICS). Cell types analysed included $CD4^+$ T-cells, $CD8^+$ T-cells, double-negative ($CD4^-CD8^-$) T-cells, natural killer (NK) cells, and invariant natural killer T (iNKT)-like cells. Each boxplot represents the median and interquartile range (IQR) of IFN-γ secreting cells with individual data points shown. Comparisons between samples with high and low background in ELISpot negative control wells were performed using the Wilcoxon rank-sum test. Statistical significance was defined as $p < 0.05$.

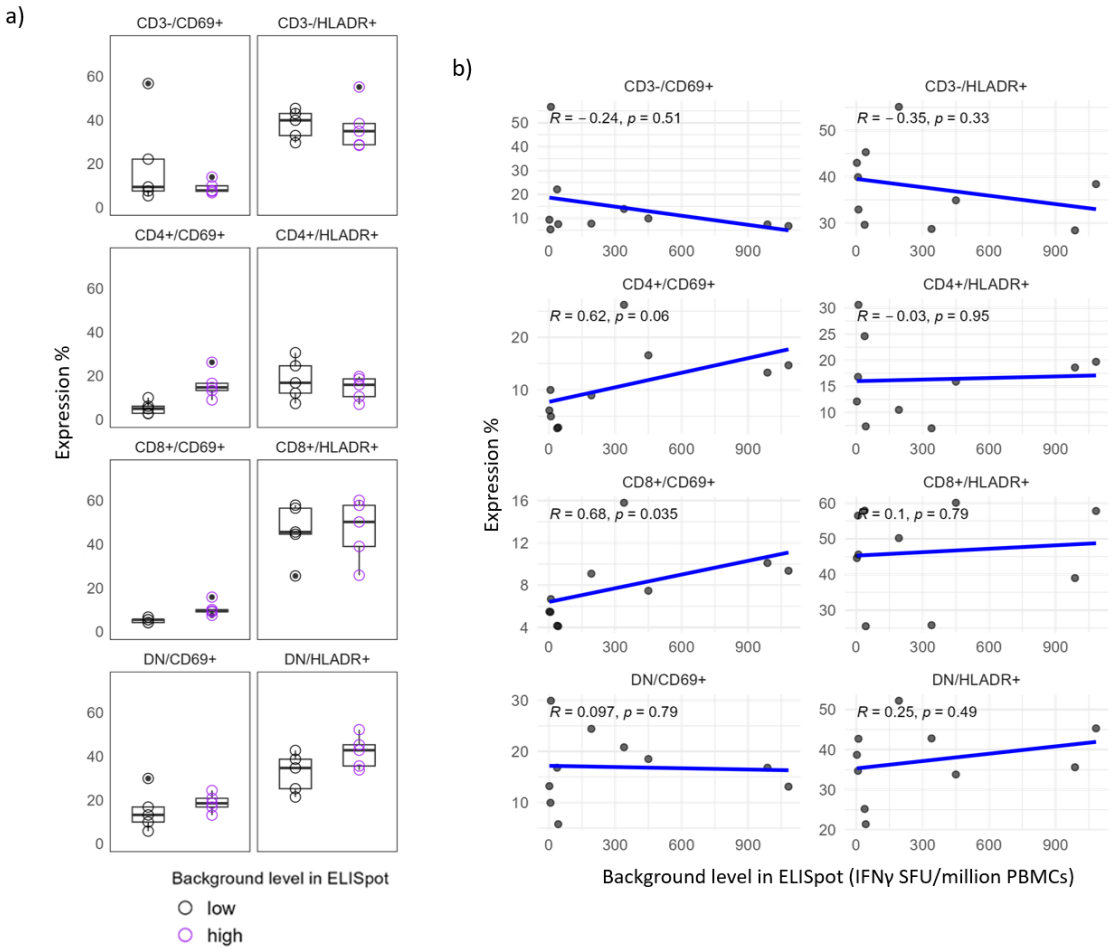

Figure 5-15 Higher levels of activated T-cells in samples with high background in ELISpot negative control wells

a) Comparison of extracellular staining result in samples with low and high background in the negative control wells of ELISpot assay. High background was defined as >50 spot-forming units (SFU) per one million PBMCs in ELISpot negative control wells. Participants (n = 5) were selected based on the availability of paired samples from the same individual, one with high background and one with low background. PBMCs were stained for CD69 and HLA-DR expression. Each boxplot represents the median and interquartile range (IQR) with individual data points shown. Paired group differences were assessed using the Wilcoxon signed-rank test. b) Correlation between ELISpot responses (SFU) and the expression percentage of activated T-cells ($CD69^+$ or $HLADR^+$) measured by flow cytometry. Correlation analysis was performed using Spearman's rank correlation test.

5.2.9 Th1 responses did not significantly differ across vaccine platforms in naïve individuals, while mRNA vaccines increased S-specific $CD4^+$ IFN-γ^+ T-cells in previously infected individuals

As shown in Section 5.2.7.1, ICS analysis revealed no significant differences in IFN-γ^+ $CD4^+$ or $CD8^+$ T-cell responses in negative-control wells between samples with low and high ELISpot background. I therefore proceeded to assess antigen-specific T-cell responses using ICS to quantify the frequencies of cytokine-producing $CD4^+$ and $CD8^+$ T-cells responding to S, M and N antigens.

In naïve individuals, no significant differences were observed between vaccine platforms in the frequencies of S-specific $CD4^+$IFN-γ^+ or $CD8^+$IFN-γ^+ T-cells post -V2 (Figure 5-16). Similarly, S-specific IL-2 and TNF production by both $CD4^+$ and $CD8^+$ T-cells did not differ across groups. No MN-specific differences in $CD4^+$ and $CD8^+$ T-cell cytokine responses were observed, noting that one individual in the viral vector group showed a $CD4^+$IFN-γ^+ response at baseline (Supplementary Figure 11). Within each vaccine group, comparisons between pre- and post-vaccination demonstrated no significant differences in the frequency of cytokine-expressing cells (Supplementary Figure 13, Supplementary Figure 14, Supplementary Figure 15).

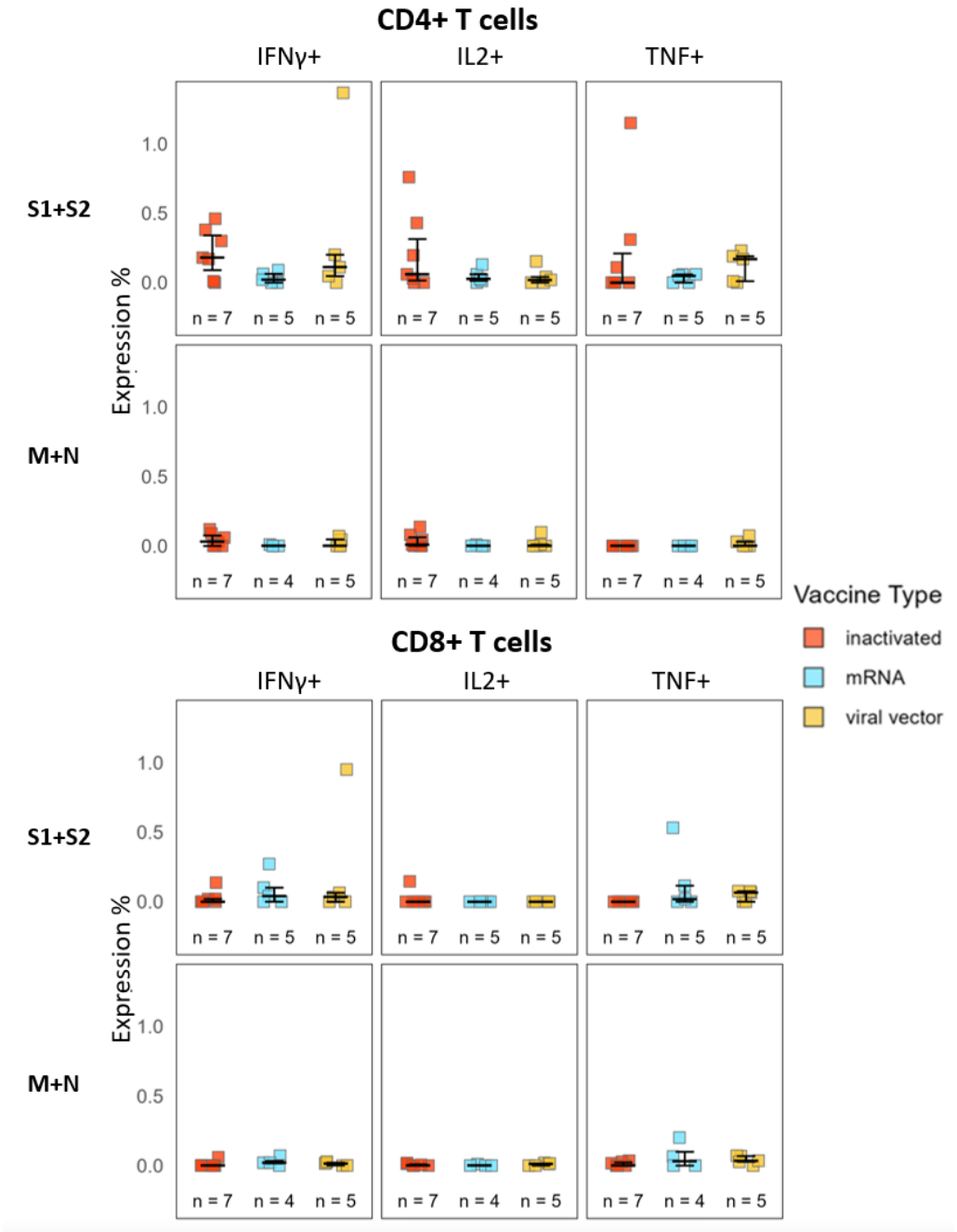

Figure 5-16 Comparison of cytokine-producing T-cells induced by different vaccine types in SARS-CoV-2 naïve individuals after two doses of COVID-19 vaccine

Peripheral blood mononuclear cells (PBMCs) from naïve individuals were stimulated with SARS-CoV-2 spike (S1+S2) and membrane and nucleocapsid (MN) for 16 hours. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons across vaccine groups were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Significance was considered at $p<0.05$.

In previously infected individuals, only the mRNA vaccine induced a significant increase in $CD4^+IFN\text{-}\gamma^+$ T-cell responses to S, compared with inactivated and viral vector vaccines (Figure 5-17). Noting that individuals in the mRNA group already exhibited detectable $CD4^+IFN\text{-}\gamma^+$ responses at baseline (Supplementary Figure 12). The longitudinal analysis of baseline and V2+28 days confirmed the significant increase of $CD4^+IFN\text{-}\gamma^+$ following mRNA vaccination (Supplementary Figure 17). In contrast, no significant changes were observed in S- or MN-specific T-cell responses, including $IFN\text{-}\gamma^+$, $IL\text{-}2^+$, or TNF^+ production by $CD4^+$ and $CD8^+$ T-cells following vaccination with viral vector or inactivated vaccines (Supplementary Figure 16, Supplementary Figure 18).

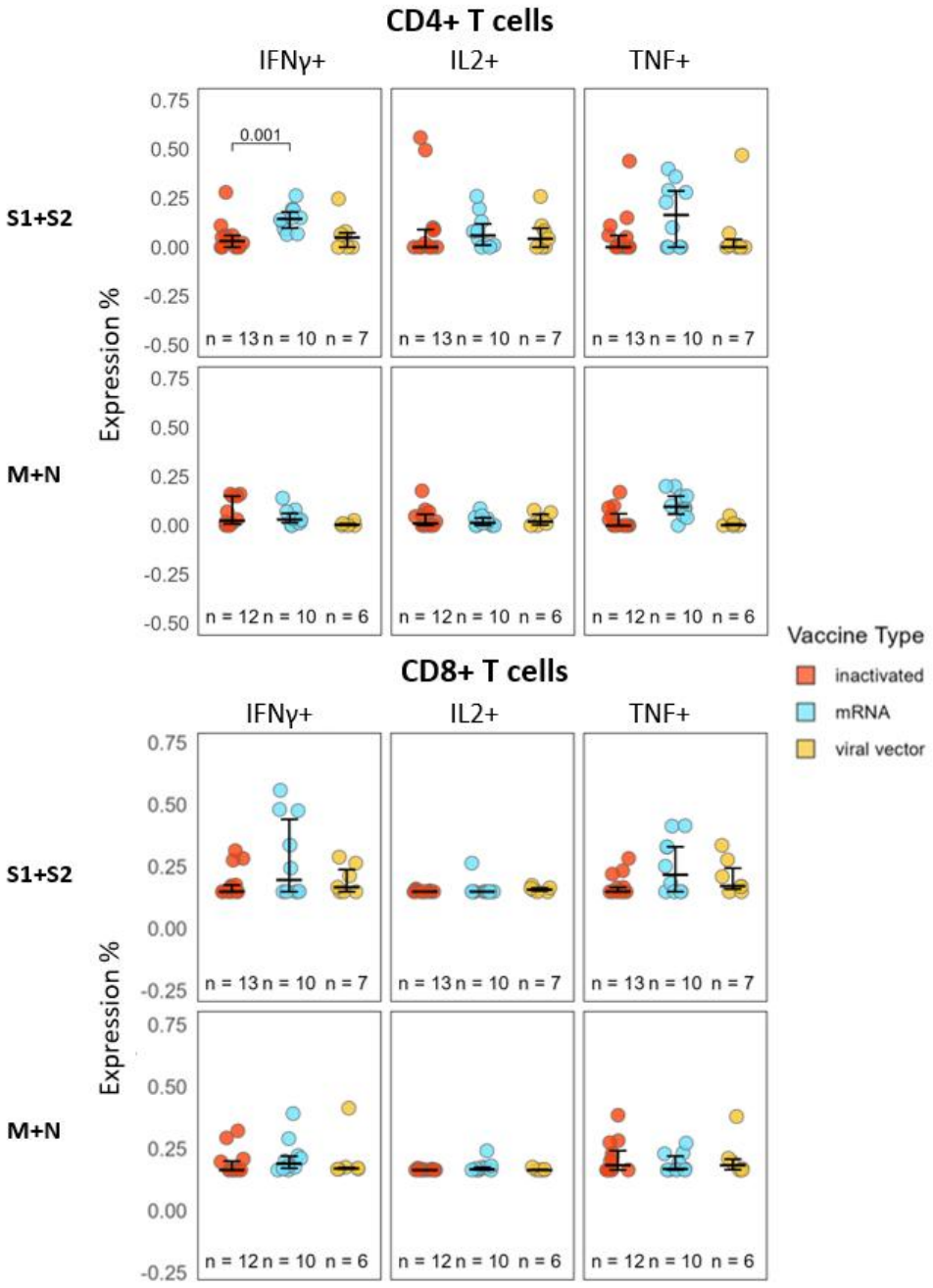

Figure 5-17 Comparison of cytokine-producing T-cells induced by different vaccine types in SARS-CoV-2 previously infected individuals after two doses of COVID-19 vaccine

Peripheral blood mononuclear cells (PBMCs) from previously infected individuals were stimulated with SARS-CoV-2 spike (S1+S2) and membrane and nucleocapsid (MN) for 16 hours. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons across vaccine groups were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Significance was considered at $p < 0.05$.test.

5.3 Discussion

This study examined how vaccine platform, prior SARS-CoV-2 infection, and pre-existing HCoV immunity shape humoral and cellular immune responses to COVID-19 vaccination in an Indonesian cohort. Overall, no significant differences in anti-S IgG or anti-S IgA were observed across vaccine platforms in SARS-CoV-2 naïve individuals. Among previously infected individuals, mRNA vaccination induced significantly higher anti-S, anti-RBD and anti-NTD IgGs and anti-RBD IgA responses compared with inactivated or viral vector vaccines. Anti-N IgG and IgA responses did not differ across vaccine platforms in either naïve or previously infected individuals. T-cell responses to spike and non-spike showed little difference between platforms in SARS-CoV-2 naïve individuals, although previously infected individuals exhibited enhanced S-specific $CD4^+$ IFN-γ^+ T-cell responses after mRNA vaccination compared with inactivated or viral vector vaccination. These findings highlight the combined influence of vaccine type and immune history in determining post-vaccination immunity.

In naïve individuals, post vaccination anti-S, anti-RBD, anti-NTD and anti-N antibody responses in serum were similar across the three platforms. In contrast, other studies have also reported lower anti-spike antibody titres after inactivated vaccines compared with mRNA or viral vector vaccines (Barin et al., 2022; Qiaoli Peng et al., 2022). The differences of these observations and the previously reported studies may be due to the small sample size of naïve individuals available in this study, reducing statistical power to detect differences post vaccination. Furthermore, because it was an observational study, participants may also have infection between vaccine doses, consistent with the observation of antibody responses to nucleocapsid post-V2 in mRNA and viral vector groups, which is unlikely induced from vaccination with spike-based vaccines. The low proportion of

nucleocapsid seroconversion in the mRNA vaccine group post-V2 may be associated with the shorter interval between vaccine doses, 21 days, compared to other vaccine groups reducing the risk of viral exposure, and the high effectiveness of mRNA vaccine (Baden et al., 2021; Polack et al., 2020). Inactivated vaccine showed a boost of N-specific IgG and IgA levels, when comparing pre- and post-vaccination results which was expected due to its component of whole virus, however there was no significant difference when comparing across vaccine platforms.

In previously infected individuals, mRNA vaccination produced the greatest increase of anti-S IgG, confirming prior reports that mRNA vaccines induce high levels of spike antigen expression and robust germinal centre activity (Lederer et al., 2022; Turner et al., 2021; Wei et al., 2022). Inactivated vaccines can also induce modest boost of non-spike-specific IgA antibody responses. However, these observations in previously infected individuals may be confounded by the circulating SARS-CoV-2 variant, influencing baseline immunity and vaccine responses.

Consistent with platform differences, logistic regression analysis identified vaccine type as a major predictor of IgG increasing, with mRNA or viral vector vaccines were associated with a significantly higher odds of increased anti-S IgG compared with inactivated vaccines. Age was also positively associated with increasing odds, whereas high pre-V1 SARS-CoV-2 S IgG was associated with lower odds of S-specific IgG increase post-V2. This inverse relationship is consistent with my observation that previously infected individuals exhibited a less pronounced increasing effect than infection-naïve individuals. This is expected because previous infection is associated with higher baseline IgG levels, limiting the range for further increases following vaccination.

A subset of participants in our cohort who were seronegative for SARS-CoV-2 nucleocapsid at baseline had antibodies to SARS-CoV-2 S. Some possible explanations include incomplete seroconversion to anti-N following infection, rapid wane of anti-N IgG compared with anti-S IgG or the presence of pre-existing cross-reactive antibodies induced by prior exposure to seasonal human coronaviruses (Anderson et al., 2021; Grifoni et al., 2020; Kaur et al., 2021; Le Bert et al., 2020; Lumley et al., 2021; Murray et al., 2023). Some participants classified as naïve in this study may have been infected before their vaccine dose, despite having no anti-N IgG. Anti-N IgG has been reported to wane faster than anti-S IgG (Lumley et al., 2021), suggesting individuals with prior SARS-CoV-2 infection may have detectable anti-S IgG despite being seronegative to anti-N IgG.

Pre-existing HCoV immunity on SARS-CoV-2 vaccine responses cannot be excluded. S2 domain of the spike protein are conserved among coronaviruses (Kaur et al., 2021; Murray et al., 2023). Multivariable logistic regression indicated that higher pre-V1 IgG to NL63 S was associated with increased odds of spike-specific IgG increasing post-vaccination. However, pre-V1 NL63-specific IgG was not associated with post-V2 anti- SARS-CoV-2 S IgG or ACE2-binding inhibition by anti-S antibodies, suggesting that NL63 antibody levels may reflect immune responsiveness rather than directly contributing to anti-S IgG increasing. Consistent with this interpretation, a previous study has shown that although NL63 and SARS-CoV-2 utilise the same ACE2 receptor for binding and entry into the host cell (Hoffmann et al., 2020), NL63 has lower binding affinity to ACE2 than SARS-CoV-2 contributing to the divergent severity of disease they cause (Brielle et al., 2020). Low affinity antibody may bind to virus with shared homology and inhibit the development of *de novo* and high affinity antibody. However, our findings do not support an inhibitory effect of pre-existing antibodies on vaccine-induced immune responses. This is in line with another study

showing that pre-existing antibodies to human coronaviruses did not interfere with the inactivated vaccine-induced neutralisation (J. Wang et al., 2021).

Th1-skewed responses are important for antiviral responses (Gil-Etayo et al., 2022). In naïve individuals, Th1 cytokine responses (IFN-γ, IL-2, TNF) to S peptides did not differ significantly pre-V1 and post-V2 vaccination and across vaccine platforms. This contrasts with several studies reporting that mRNA and viral vector vaccines induced robust Th1-skewed T-cell responses following two doses (Lim et al., 2022; Mok et al., 2022; Sahin et al., 2021; Swanson et al., 2021). Inactivated vaccines predominantly induce $CD4^+$ T-cells, while mRNA vaccine can generate both $CD4^+$ and $CD8^+$ T-cells (Lim et al., 2022). I did not observe an increase in anti-N T-cell responses in our cohort; however, previous work has shown that inactivated vaccines can induce N-specific T-cells and that these responses are better preserved against variants such as Omicron than those directed at S or M (Lim et al., 2022). By contrast, among previously infected individuals, mRNA vaccination significantly increased S-specific $CD4^+$IFN-γ^+ T-cells, whereas viral vector and inactivated vaccines did not, suggesting that the mRNA platform may more effectively augment pre-existing T-cell memory than the other vaccine platforms.

Unexpectedly high background responses in ELISpot assays prompted additional analysis of potential cellular contributors. Samples with high background exhibited increased frequencies of IFN-γ^+ NK cells and correlation of $CD8^+CD69^+$ T-cells in unstimulated IFN-γ spot formation. These findings are consistent with cytokine-driven and antigen-independent activation. PAMPs present due to potential contamination can activate monocytes, which leads to cytokine secretion e.g. type I IFN-s, IL-12, IL-15 which in turn can cause IFN-γ production in NKs and bystander activation of $CD8^+$ T-cells in the absence of antigen as described in several studies (Kohlmeier et al., 2008; Maurice et al., 2019; Seo

et al., 2021; Soudja et al., 2012). In addition, I could not exclude the possibilities of technical reasons during blood collection, processing or storage that may affect the quality of the samples for cellular analysis (Browne et al., 2024; Hope et al., 2021).

To assess whether baseline factors related to geographical location influence immune response differences, a comparative analysis was performed between the UK and Indonesian cohorts. Unvaccinated individuals from the Indonesian cohort exhibited higher baseline antibody titres to seasonal coronaviruses compared with those from the UK cohort. This likely reflects differences in infection burden at the time of sample collection, given that Indonesian samples were collected later in the pandemics. Despite known differences in HLA distribution between the UK and Indonesian populations, no significant differences were observed in vaccine-induced T-cell responses among either SARS-CoV-2 naïve or previously infected individuals who received mRNA vaccines. This finding suggest that mRNA vaccination induces comparable T-cell responses across populations.

Limitations

Limitations of this study include the relatively small sample sizes in some vaccine groups, particularly the viral vector group and the samples for cellular analysis, which constrained statistical power for data analyses. Some participants may have experienced asymptomatic, unrecognised SARS-CoV-2 infection between their pre-V1 and post-V2 visit. Although I only selected participants without a recorded infection between vaccine dose, the increase of IgG to N post vaccination suggests potential unconfirmed infections to SARS-CoV-2. In addition, I cannot exclude unmeasured differences between individuals such as socio-economic status, occupational exposure to the virus, and different timings with respect to surges in community infection rates which affect vaccine response observed in this

observational study. In my multivariable logistic regression model, the large effect size observed for baseline anti-NL63 spike IgG should be interpreted cautiously, and may reflect the right-skewed distribution of baseline NL63 S IgG titres, with relatively few observations at higher values. Larger studies will be required to refine this estimate.

Future work could incorporate live-virus neutralisation assays with complement restoration, which is more representative of neutralisation activity (Mellors et al., 2025) to provide insight into differences across vaccine platforms which may have been missed by conventional neutralisation assays. Further investigation of the $CD4^{-}CD8^{-}$ (double-negative, DN) T-cell population, which showed elevated but not statistically significant IFN-γ secretion in unstimulated wells, may also identify the cellular sources contributing to high background in ELISpot assay, such as MAIT or γδ T-cell subsets, which are known to secrete IFN-γ.

Conclusions

In conclusion I found some evidence that vaccine platform, prior SARS-CoV-2 infection, and pre-existing HCoV immunity together shape COVID-19 vaccine responses. The increased capacity of mRNA vaccines to induce immune responses to spike supports their use in individuals with prior infection and in booster programmes. Inactivated vaccine induced immune responses to nucleocapsid, but in this small cohort I was unable to detect significant differences across vaccine groups. The influence of pre-existing immune responses needs to be accounted when evaluating vaccine immunogenicity. Understanding these interactions is essential for optimising vaccine strategies and anticipating population level immunity in settings with diverse viral exposure histories.

6 General discussion

Vaccination is one of the most effective ways to reduce the global burden of infectious diseases, including SARS-CoV-2. COVID-19 vaccines have been effective in preventing severe disease and mortality, but the immune responsiveness varies between individuals (Berber & Ross, 2024; Falahi & Kenarkoohi, 2022; Nehar-Belaid et al., 2023). Some individuals develop robust and durable immune responses, while others, despite being immunocompetent, show weaker or more short-lived immunity following vaccination and experience breakthrough infections. This heterogeneity raises scientific and public health questions about the mechanisms that shape vaccine-induced protection.

This thesis examines immune responsiveness following diverse COVID-19 vaccine platforms in real-world settings. The findings demonstrate that vaccine-induced immune responses vary between healthy individuals shaped not only by vaccine platforms, but also by prior antigenic exposure and host immune environment.

My first results chapter characterised immune responses in UK HCWs who self-reported no prior SARS-CoV-2 infection following vaccination with mRNA or viral vector vaccines. Individuals without known breakthrough infection exhibited robust spike-specific antibody responses in both systemic and mucosal compartments, as well as detectable spike-specific T-cell responses, consistent with high immunogenicity of COVID-19 vaccine used in the vaccination program. The limited mucosal immunity induced by intramuscular vaccination, however, highlights the potential value of mucosal vaccine strategies to enhance immune protection at the primary site of viral entry and transmission. Non-spike immune responses and localised antibody responses were detected in many healthcare workers who reported

no prior infection suggests that hybrid immunity is more common than self-reported infection histories indicate.

Pre-existing immunity to seasonal coronaviruses likely plays a limited role in enhancing vaccine-induced immunity. The associations between HCoV S-specific antibodies and vaccine responses were moderate. Similarly, the T-cell responses to RTC, which were conserved across coronaviruses, were also limited. These findings suggest that the immune profiles observed among UK healthcare workers between May 2022 and April 2023 are more likely explained by unrecorded SARS-CoV-2 exposure sustaining vaccine-induced immunity rather than protective pre-existing HCoV immunity. Nevertheless, some individuals may have mounted durable vaccine-induced responses in the absence of infection, indicating heterogeneity in immune responses even among similarly vaccinated individuals.

The second results chapter advances the understanding that vaccine-induced T-cell responses are shaped by baseline host immune competence. Proteomic profiling of pre-second dose vaccine samples was used to identify protein markers associated with T-cell responses following mRNA vaccination. Multivariable analysis revealed that protein markers associated with immune ageing, inflammatory regulation, and extracellular matrix remodelling, together with age, sex, and CMV serostatus, were predictive of T-cell responsiveness. These findings support the development of a proof-of-concept immune competence score, which has the potential to be used as a translational tool for identifying inter-individual variation in vaccine-induced responses and informing more tailored vaccination strategies. SKAP2, in particular, was detectable using a clinically accessible assay ELISA and showed moderate agreement between proteomic measurements, highlighting its potential as a candidate for further translational evaluation.

Immunophenotypic analysis further provided insight into underlying cellular phenotypes for T-cell vaccine responsiveness following mRNA vaccination among healthy individuals. T-cell response group demonstrated a more preserved naïve and memory T-cell compartment and more regulatory T-cells one month after vaccination, consistent with a functionally competent T-cell response. In contrast, T-cell non-response group exhibited markers associated with senescence and functional exhaustion, including increased frequencies of terminally differentiated effector memory T-cells, reduced naïve T-cell proportions, and elevated expression of inhibitory or exhaustion-associated markers. These results showed that host immune competence should be accounted in vaccine design and vaccination programmes, such as formulations that enhance T-cell priming and expansion, the use of additional booster doses, or adjustment to vaccine intervals.

My third results chapter showed that immune responses differed in magnitude and breadth across inactivated, viral vector, and mRNA vaccines and were influenced by baseline immunity defined by prior SARS-CoV-2 infection status and pre-vaccination seasonal coronaviruses spike-specific IgG levels. Consistent with previous studies, mRNA and viral vector vaccine was associated with strong spike-specific humoral and cellular immune responses, and inactivated vaccines elicited broad responses that included non-spike-specific immunity compared with spike-based vaccines. However, these observations were only apparent in previously infected individuals and when comparing pre- and post-vaccination timepoints within individuals and was not powered to observe differences between vaccine platforms in infection-naïve individuals. The strong boosting effect of mRNA vaccines in previously infected individuals supports the use of mRNA platforms in vaccine booster strategies, particularly in populations with high levels of prior exposure. In addition, incorporating multiple viral antigens, such as inactivated vaccines, may contribute

to broader immune recognition and could offer advantages against highly mutated viral variants.

Limitations

While this thesis provides new insight into factors associated with SARS-CoV-2 vaccine responsiveness, several limitations should be acknowledged. First, although some elements of the study design were prospective, analyses were largely observational and focused on immune responses at defined vaccination time points. Consequently, the study could not fully characterise longer-term changes in immune responses, including immune waning beyond day 28 and responses to subsequent booster doses. Extended longitudinal follow-up would provide a more complete picture of immune durability. Second, sample sizes in some patient groups were small. This limited statistical power to detect differences between vaccine groups or to validate the prediction model. Third, there was a bias of age and sex, given the predominance of women in UK HCW study and the fact that they are individuals of working age. Future studies incorporating larger cohorts with more age diverse cohort, a more balanced sex distribution, and broader inclusion beyond healthcare worker populations will be required to confirm COVID-19 vaccine responsiveness findings.

Further work could include higher resolution approaches to comprehensively characterise immune heterogeneity and functional immunity. In this study, a broad immunophenotyping panel encompassing 77 markers was generated, however, due to time constraints, analyses were focused on targeted immune features most relevant to vaccine-induced T-cell responsiveness. Expanded and unbiased analysis of this dataset could further identify both shared and distinct immune cell features between T-cell response group and non-response group, as well as across healthy and immunocompromised individuals. Single-cell

transcriptomic profiling would enable exploration of immune activation pathways that lead to differential immune responsiveness across vaccine platforms and between infection-naïve and previously infected individuals. I did not perform single cell analysis for the Indonesian cohort as initially planned, due to the limited availability of PBMC samples of sufficient quality to do this analysis. In addition, the assessment of antibody neutralisation function using live-virus neutralisation assays with complement restoration could provide further understanding of diverse vaccine platforms (Mellors et al., 2025), as the standard assay heat inactivates complement in the serum or plasma, which underestimates the neutralisation activity, particularly for inactivated vaccines that can induce IgM and complement activity. Moreover, further work to validate candidate markers such as SKAP2 in larger and independent cohorts, and longitudinal assessment to determine whether pre-vaccination protein levels predict durability of vaccine-induced immunity, as well as functional studies examining the relationship between circulating SKAP2 with myeloid cell activity and T-cell priming may further clarify its role as a marker of immune competence rather than a direct mediator of vaccine responsiveness.

Conclusions

This research contributes to the growing evidence that immune responsiveness following vaccination is heterogeneous, even among healthy adults. By examining systemic and mucosal antibody responses, cellular, and proteomic analyses, this work supports that COVID-19 vaccination induce humoral and cellular immune responses in most healthy individuals, and these responses were shaped by baseline immune state, exposure history and vaccine platforms.

These findings have the potential to inform the optimisation of vaccination strategies against SARS-CoV-2 and potentially other viral pathogens. Baseline host immune competence, including the capacity to mount effective innate and adaptive immune responses, plays an important role in determining vaccine responsiveness. Immune ageing and chronic inflammatory states associated with ageing or persistent infections may contribute to reduced vaccine-induced T-cell responses. In contrast, pre-existing immunity to seasonal human coronaviruses appears to play only a limited role in protecting against SARS-CoV-2 infection. Individuals with reduced immune competence may require additional booster doses or optimisation of vaccine intervals to achieve adequate protection. In addition, vaccine design and booster strategies should consider approaches that enhance immune protection in high-transmission settings, including the use of mRNA vaccines as boosters, the incorporation of multiple viral antigens, and the induction of immune responses at the site of viral entry to reduce the transmission of highly mutated SARS-CoV-2 variants.

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8 Declaration

During the final preparation stages of this thesis, AI-assisted tools were used to support proofreading, grammar correction, and improvements to clarity of expression, but not for content generation. Responsibility for the content of this thesis rests entirely with the author.

9 Publications

No publications have arisen yet from the work presented in this thesis. The author is part of the SEACOVARIANT consortium which co-authored the following manuscripts:

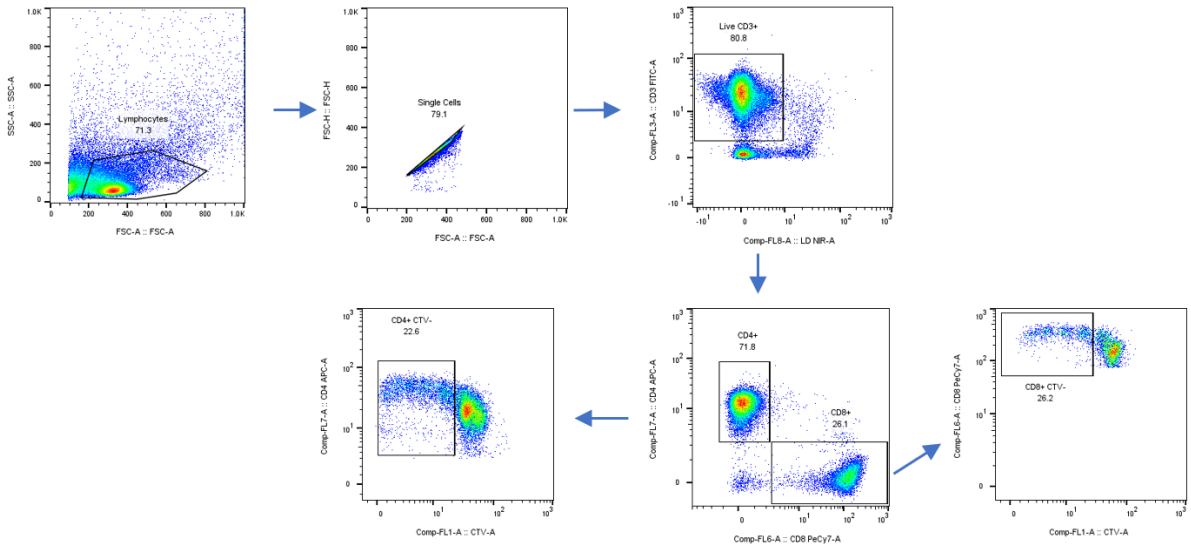
Poolchanuan, P., Tiacharoen, V., Dulsuk, A. *et al.* Longitudinal analysis of neutralizing antibodies against SARS-CoV-1 and different SARS-CoV-2 strains in breakthrough and unvaccinated COVID-19 patients in Thailand (2021-2022). *Sci Rep* (2026). https://doi.org/10.1038/s41598-025-33388-7

Poolchanuan, P., Matsee, W., Dulsuk, A. *et al.* Temporal correlations between RBD-ACE2 blocking and binding antibodies to SARS-CoV-2 variants in CoronaVac-vaccinated individuals and their persistence in COVID-19 patients. *Sci Rep* **15**, 15831 (2025). https://doi.org/10.1038/s41598-025-98627-3

Suwarti, S., Lazarus, G., Zanjabila, S. *et al.* Anti-SARS-CoV-2 antibody dynamics after primary vaccination with two-dose inactivated whole-virus vaccine, heterologous mRNA-1273 vaccine booster, and Omicron breakthrough infection in Indonesian health care workers. *BMC Infect Dis* **24**, 768 (2024). https://doi.org/10.1186/s12879-024-09644-y

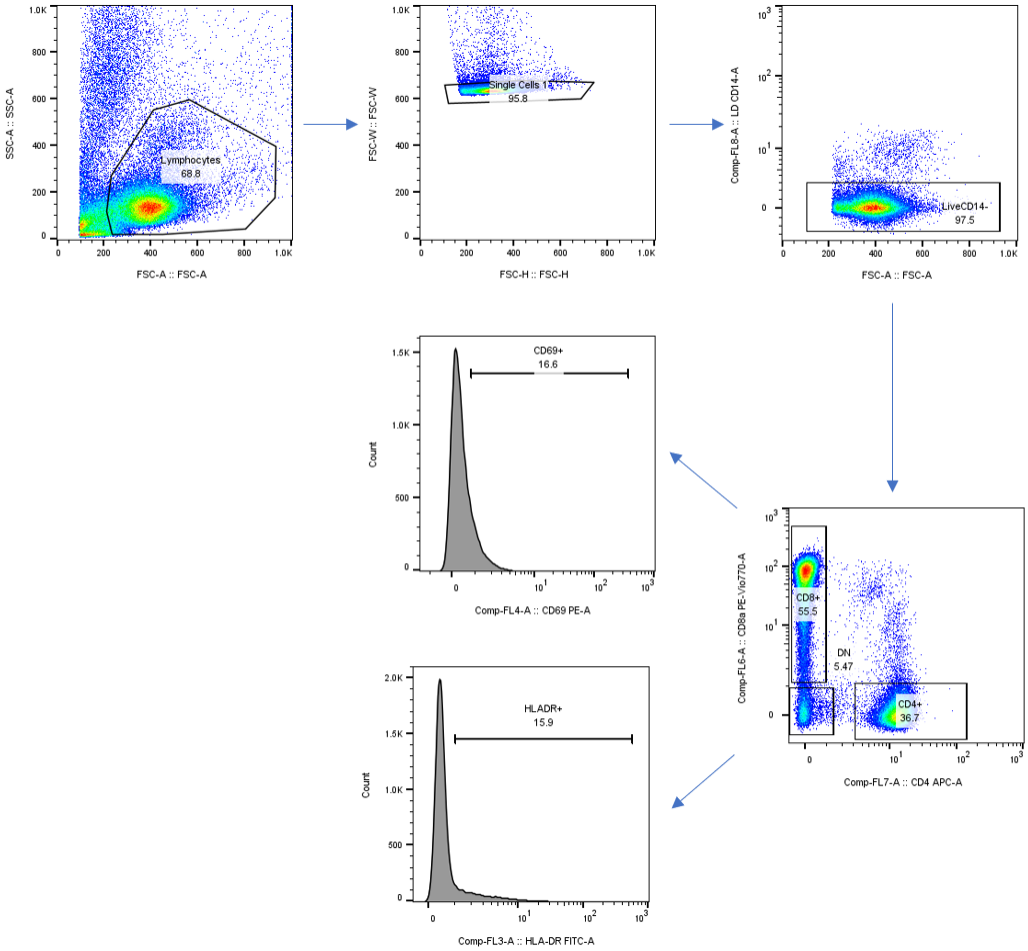
10 Appendices

10.1 Supplementary figures



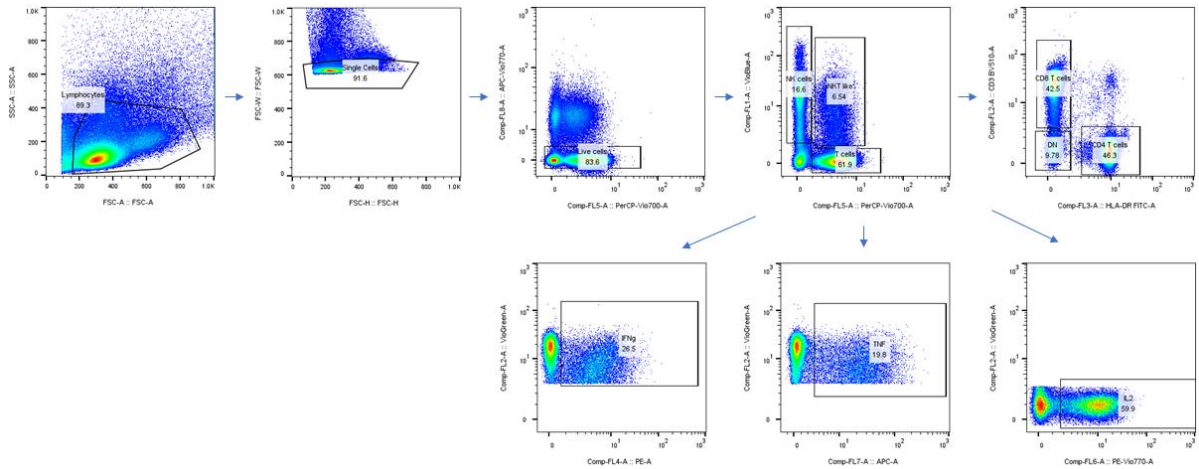
Supplementary Figure 1 Gating strategy for T-cell proliferation assays

PBMC was labelled with Cell Trace Violet and stimulated with SARS-CoV-2 peptides (S1, S2, M, N, NSP3b, NSP7-11, NSP12a, NSP12b, NSP13 and ORF3) for 7 days at 37°C. Cells were then stained using CD3 FITC, CD4 APC, CD8a PE/Cyanine7 and L/D Near Infra-Red. First, lymphocytes were gated on FSC vs SSC. Live T-cells were then gated on CD3 vs live dead stain. CD4 and CD8 were then gated. CD4 proliferation was gated on CD4 vs Cell Trace Violet. Similarly, CD8 proliferation was gated on CD8 vs Cell Trace Violet. The proportion of proliferation was analysed using FlowJo.



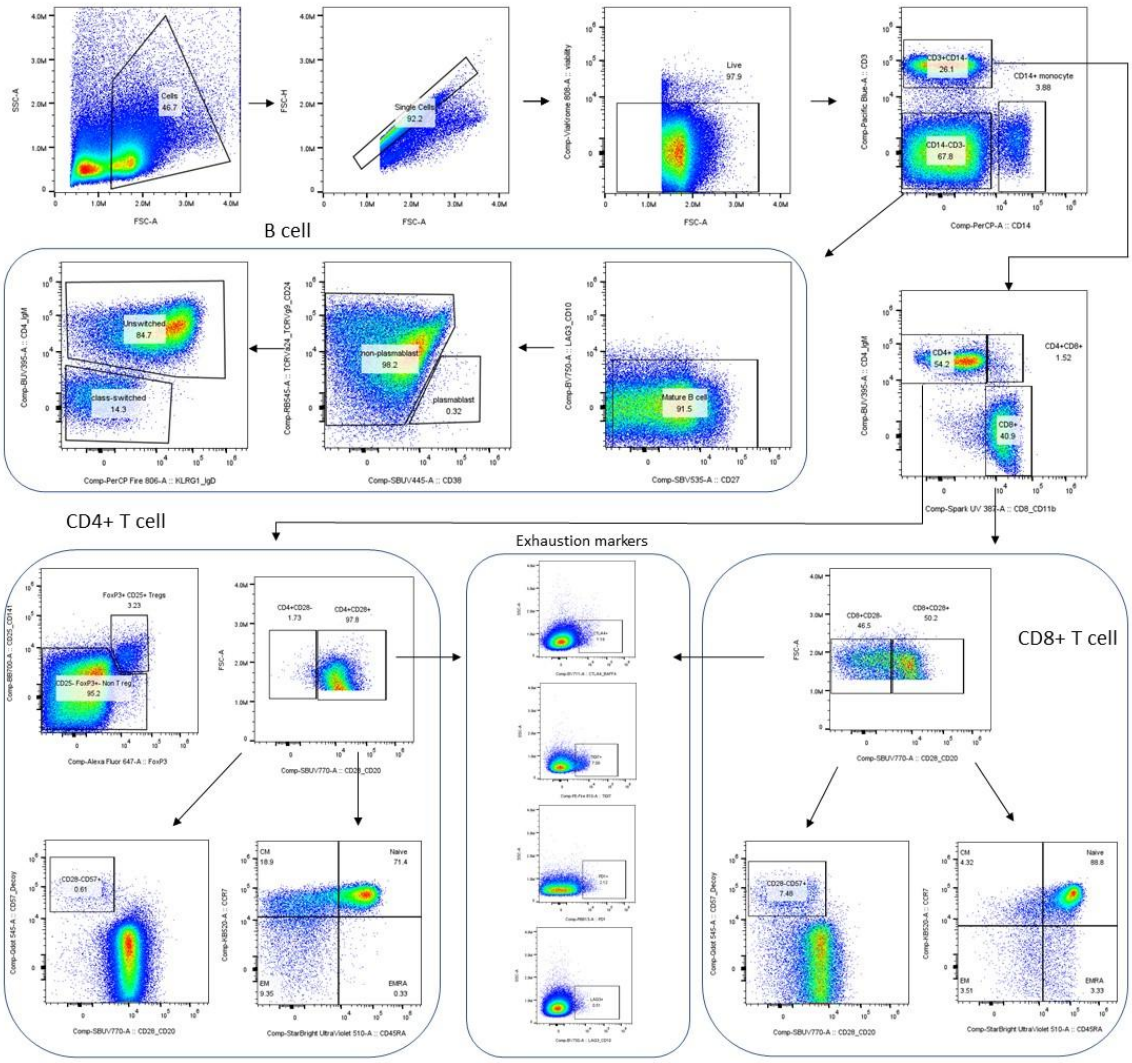
Supplementary Figure 2 Gating strategy for extracellular staining flow cytometry

PBMCs were then stained using CD3 BV510, CD4 APC, CD8a PE-Vio770, CD56 BV421, CD69 PE, HLA-DR FITC and L/D Near Infra-Red. First, lymphocytes were gated on FSC vs SSC. Live T-cells were then gated on CD14- vs live dead stain. CD4+, CD8+ T cells and CD4-CD8- (double negative (DN)) cells were then gated. Each cell was gated against CD69+ and HLADR+. The proportion of cells was analysed using FlowJo.



Supplementary Figure 3 Gating strategy for intracellular staining flow cytometry

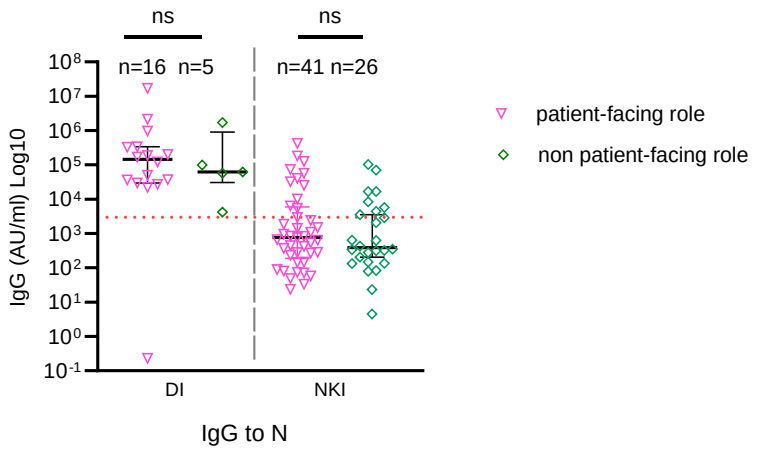
PBMC was stimulated with SARS-CoV-2 peptides (S1+S2, M+N) for 16 hours at 37ºC. Cells were then stained using CD3 PerCP, CD4 FITC, CD8a BV510, CD56 BV421, IFN-γ PE, TNFα APC, IL2 PE-Cy7 and L/D Near Infra-Red. First, lymphocytes were gated on FSC vs SSC. Live T-cells were then gated on CD3 vs live dead stain. NK cell, iNKT like cell and T-cells were then gated. Each cell was gated against IFN-γ, IL2 and TNFα. The proportion of cytokine secretion was analysed using FlowJo.



Supplementary Figure 4 Gating strategy for immunophenotyping using flow cytometry

Peripheral blood mononuclear cells (PBMCs) were stained and analysed by flow cytometry. Initial gates were set on FSC vs SSC to identify cells, followed by gating on FSC-A vs FSC-H to select single cells. Live cells were identified using a viability dye. Monocytes were excluded by the presence of CD14. CD3–CD14– cells were classified as B cells and mature B cells were defined as CD10-CD27+/. Non-plasma blasts were identified by CD38+ CD24+/-. Non-plasma blasts were further differentiated into class-switched (IgM-IgD-) and non-class-switched. CD3+ T-cells were further gated into CD4+ and CD8+. T-cell differentiation states were classified using CD45 and CCR7 markers. Loss of CD28 expression was assessed within both CD4+ and CD8+ T-cells. Senescent T-cells were defined by CD28-CD57+ and exhausted T-cell markers: PD-1, CTLA4, LAG3 and TIGIT expression. Regulatory T-cells were identified using FoxP3 and CD25 from CD4+ T-cells. The proportion of cell subsets were quantified using FlowJo.

A)

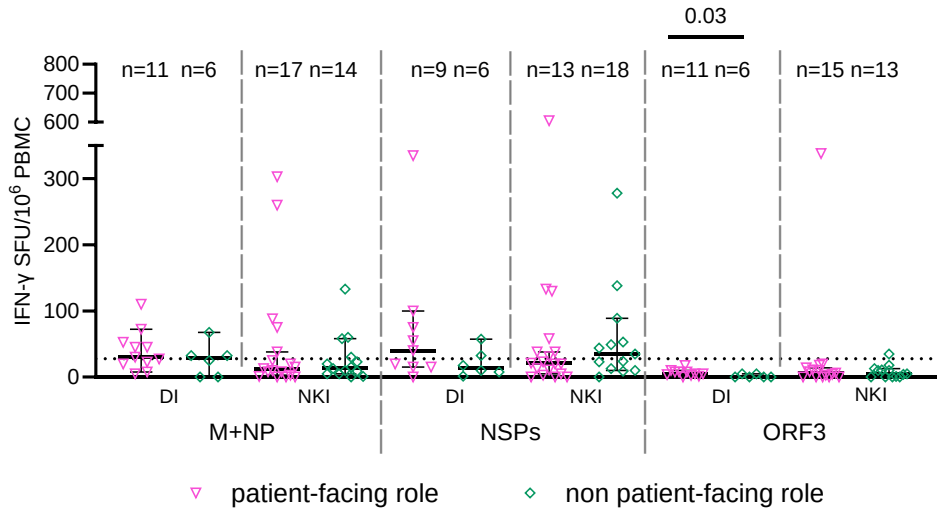


IgG to N

	Seropositive	Seronegative	Total
Patient-facing role	11	29	40
Non patient-facing role	6	17	23
Total	17	46	63

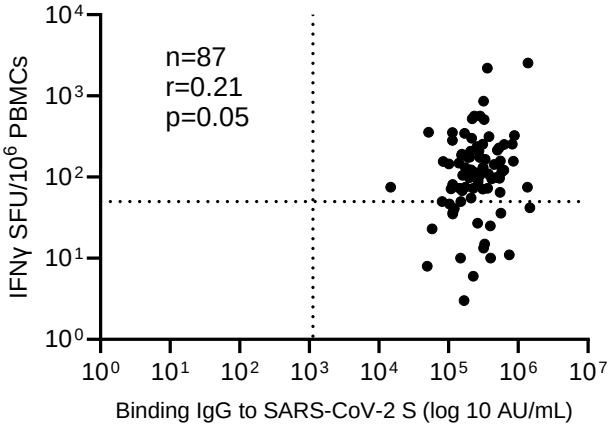
Fisher's exact test
The two-tailed P value equals 1.0000

B)



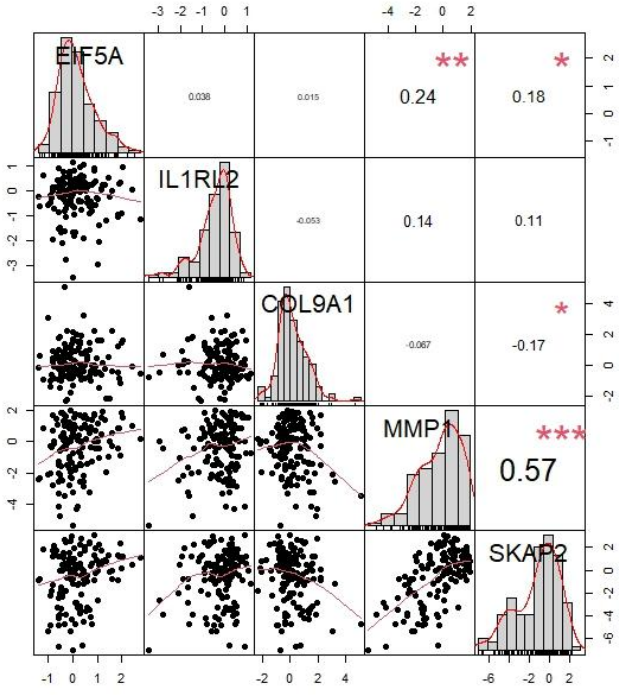
Supplementary Figure 5 Comparison of IgG responses to SARS-CoV-2 nucleocapsid and IFN-γ secreting cell numbers to non-spike between HCWs with patient-facing role and non-patient-facing role

A) IgG response to N in plasma or serum and B) T-cell response as measured by ELISpot to M+NP, NSPs and ORF3. Each dot represents data from each sample. Error bar is median with 95% CI. Pink inversed triangle represents those with patient-facing job. Green diamond represents those with non-patient facing role. Comparison between two groups was measured using Wilcoxon rank-sum test. Dotted line represents positive cut-off. Fisher's test was also used to compare proportion between groups. DI=Detected infection; NKI=No Known Infection.



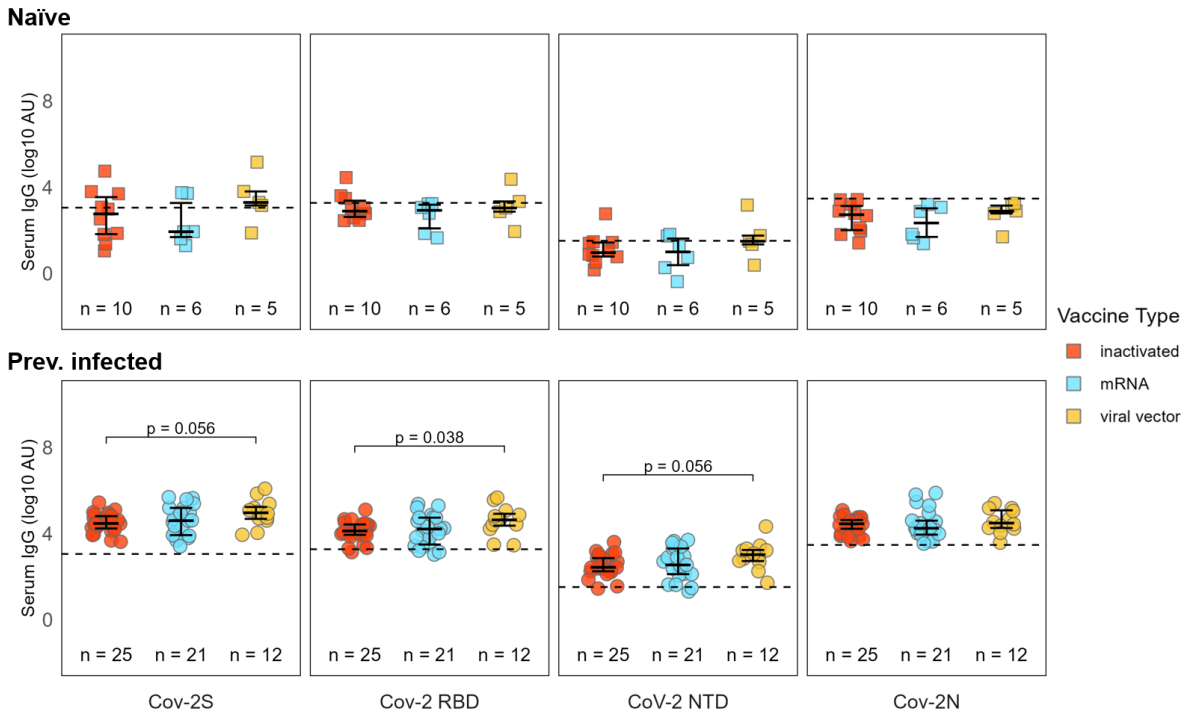
Supplementary Figure 6 Correlation of IgG to SARS-CoV-2 spike and IFN-γ secreting cells to spike

Antibody and T-cell responses to SARS-CoV-2 detected in blood a month after two doses of BNT162b2 with 6-17 weeks interval between doses. Dashed lines are positive cut-offs. Correlation analysis is performed using Spearman's test.



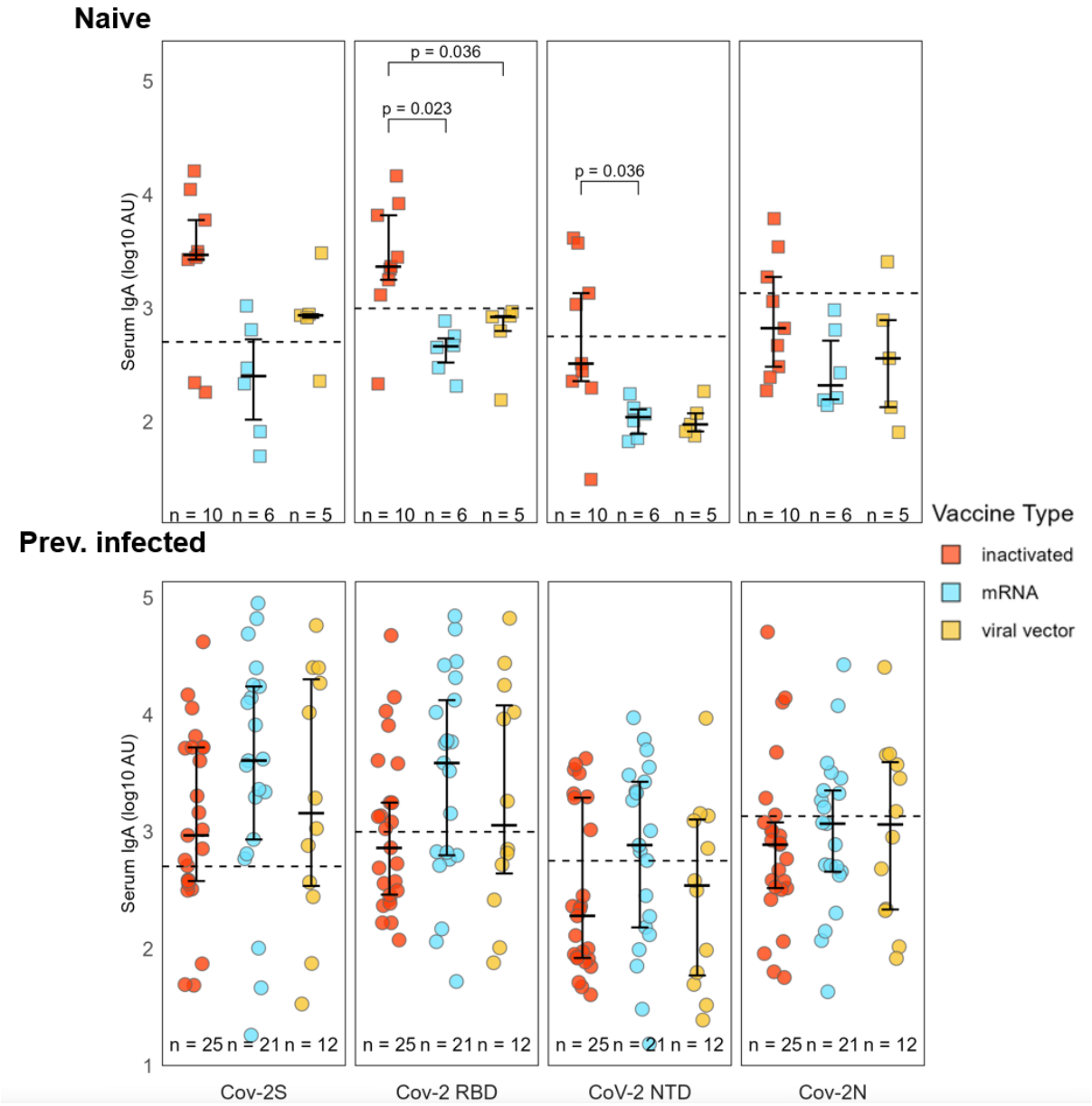
Supplementary Figure 7 Correlation between predictive protein markers as identified by LASSO selection

The correlation of protein markers, as selected using least absolute shrinkage and selection operator (LASSO), were analysed using Spearman's. *=P<0.05, **=P<0.01, ***=P<0.001.



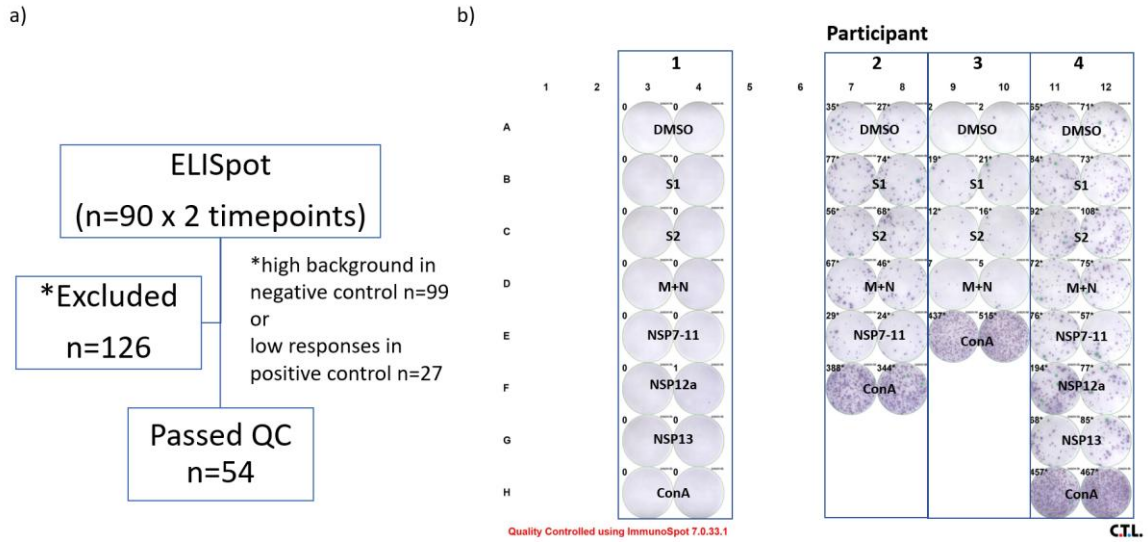
Supplementary Figure 8 Pre-V1 IgG levels to SARS-CoV-2 antigens across different vaccine groups

IgG responses to SARS-CoV-2 spike, RBD, NTD and nucleocapsid (N) proteins before first dose of COVID-19 vaccine are shown for each vaccine groups. Dashed line represents seropositivity cut-off value for each antigen-specific antibody. Group comparisons were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Error bar represent the median and interquartile range (IQR), with individual data points shown.



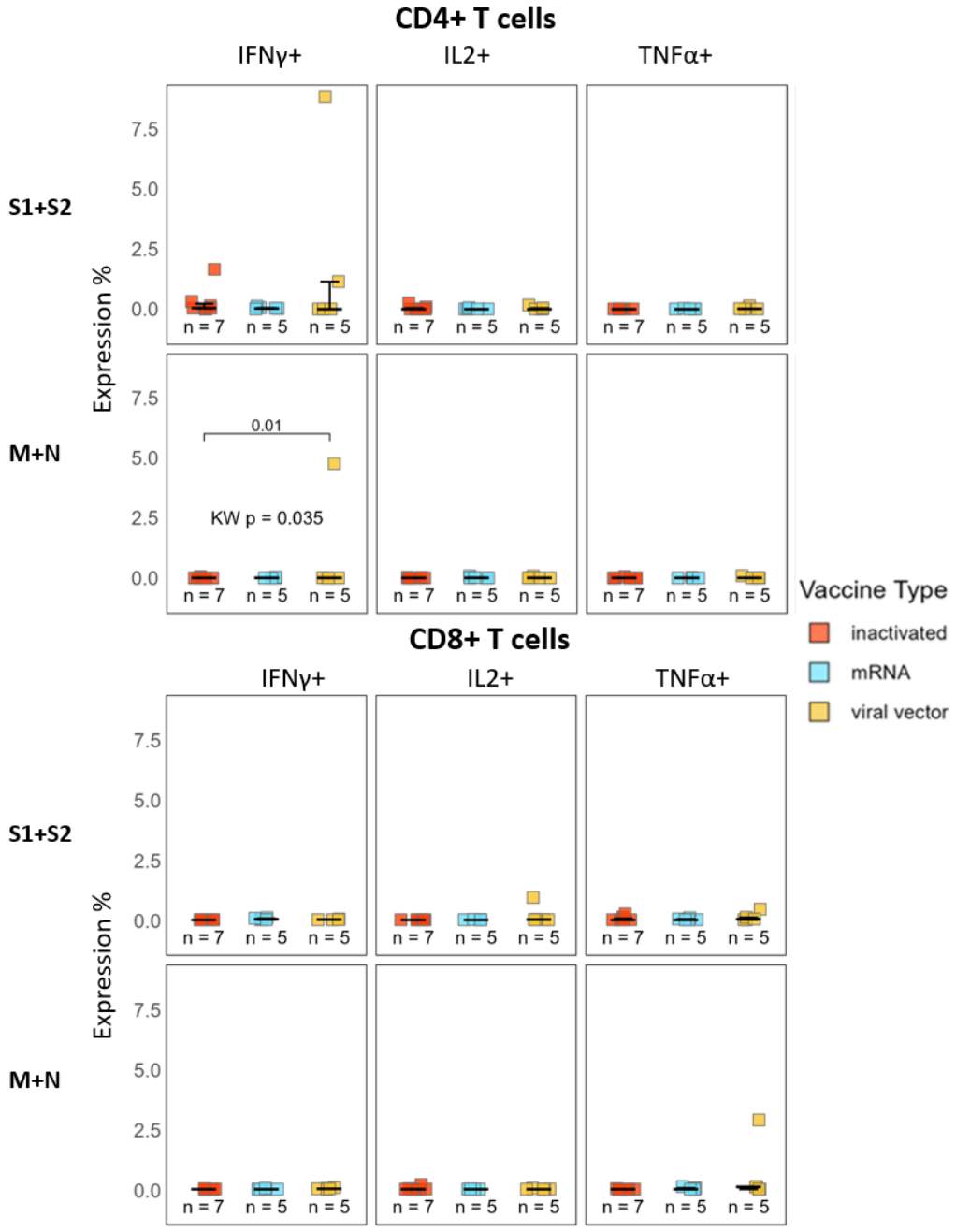
Supplementary Figure 9 Pre-V1 IgA levels to SARS-CoV-2 antigens across different vaccine groups

IgA responses to SARS-CoV-2 spike (S) and nucleocapsid (N) proteins before first dose of COVID-19 vaccine is shown for each vaccine groups. Dashed line represents seropositivity cut-off value for each antigen-specific antibody. Group comparisons were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Error bar represent the median and interquartile range (IQR), with individual data points shown. Significance was considered at $p < 0.05$.



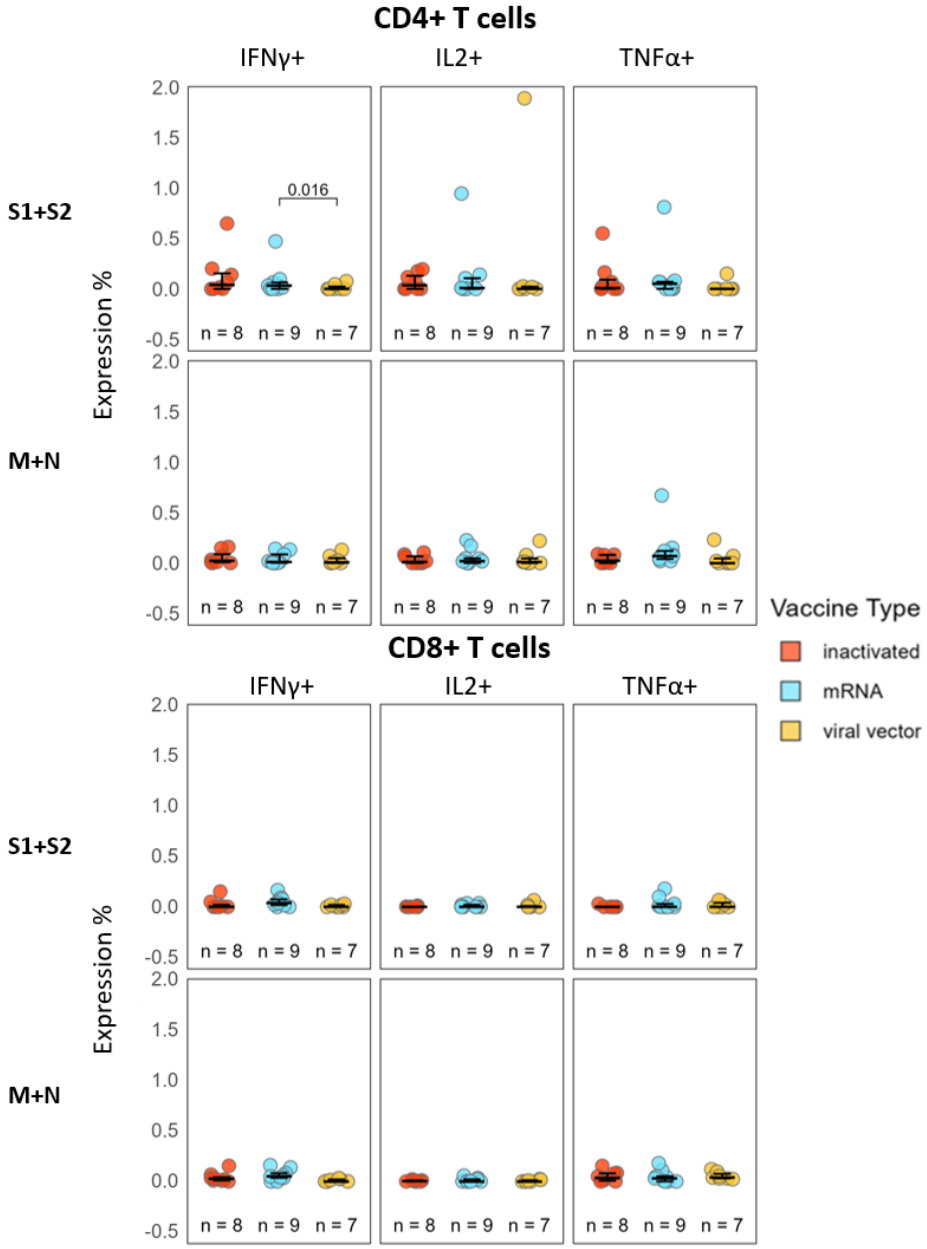
Supplementary Figure 10 Overview of ELISpot assay quality control outcomes and representative image of an ELISpot plate

a) Overview of all samples processed using the ELISpot assay, including the number of samples excluded due to high background in negative control wells and those that passed quality control for inclusion in statistical analysis. High background was defined as samples with >50 spot forming unit (SFU) per one million PBMCs in negative control wells. Low response in positive control wells was defined as <200 SFU/1mio PBMCs. b) Representative image of an ELISpot plate showing negative control well (DMSO), positive control well (ConA) and SARS-CoV-2 antigen stimulated wells. Each sample was plated in duplicate.



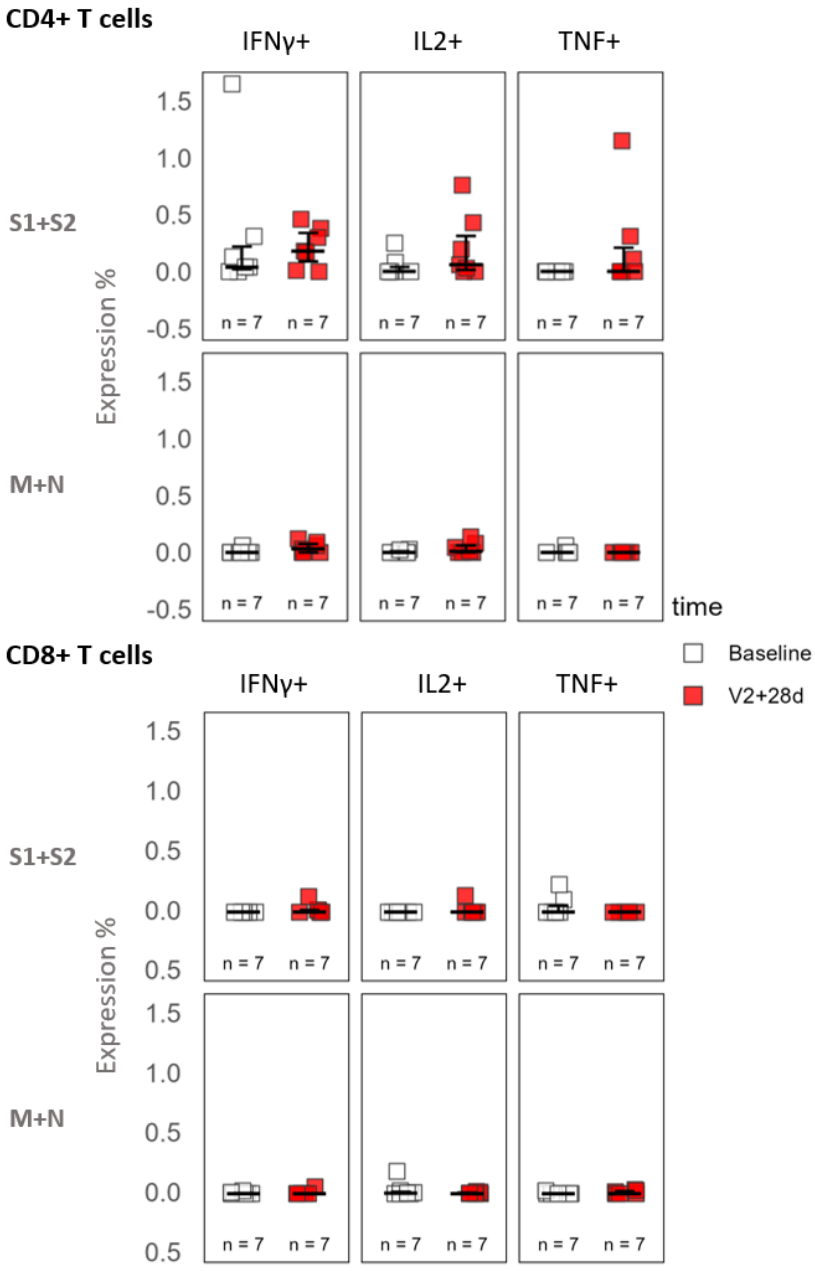
Supplementary Figure 11 Comparison of cytokine-producing T-cells induced by different vaccine types in SARS-CoV-2 naïve individuals at pre-V1

Peripheral blood mononuclear cells (PBMCs) from naïve individuals were stimulated with SARS-CoV-2 spike (S1+S2) and membrane and nucleocapsid (MN) for 16 hours. Participants were classified as SARS-CoV-2 naïve based on seronegative to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons across vaccine groups were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Significance was considered at $p < 0.05$.test.



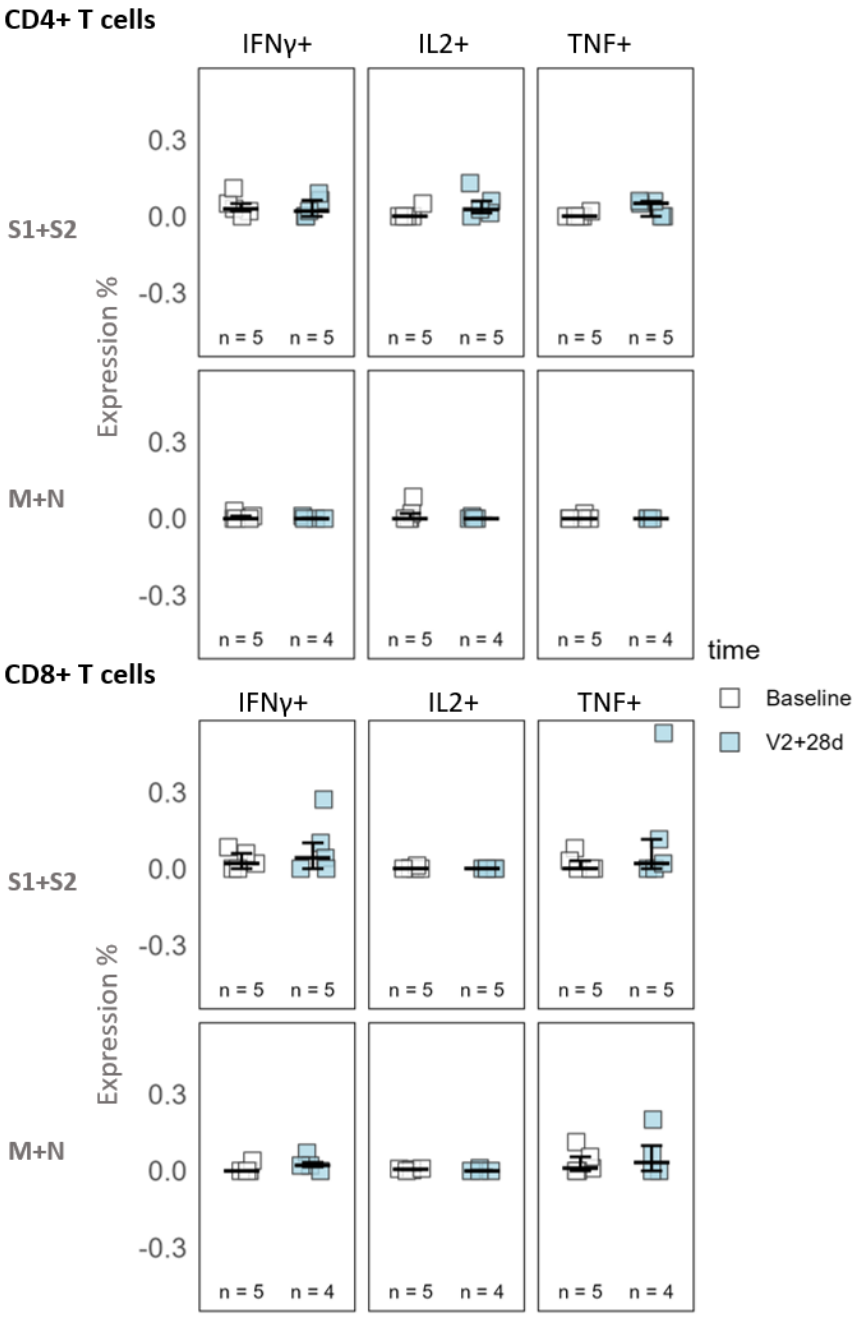
Supplementary Figure 12 Comparison of cytokine-producing T-cells induced by different vaccine types in SARS-CoV-2 previously infected individuals at pre-V1

Peripheral blood mononuclear cells (PBMCs) from previously infected individuals before their first dose of COVID-19 vaccine were stimulated with SARS-CoV-2 spike (S1+S2) and membrane and nucleocapsid (MN) for 16 hours. Participants were classified as SARS-CoV-2 previously infected based on seropositivity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons across vaccine groups were performed using the Kruskal-Wallis (KW) test, followed by post hoc Dunn's test with Bonferroni correction for multiple comparisons. Significance was considered at $p < 0.05$.test.



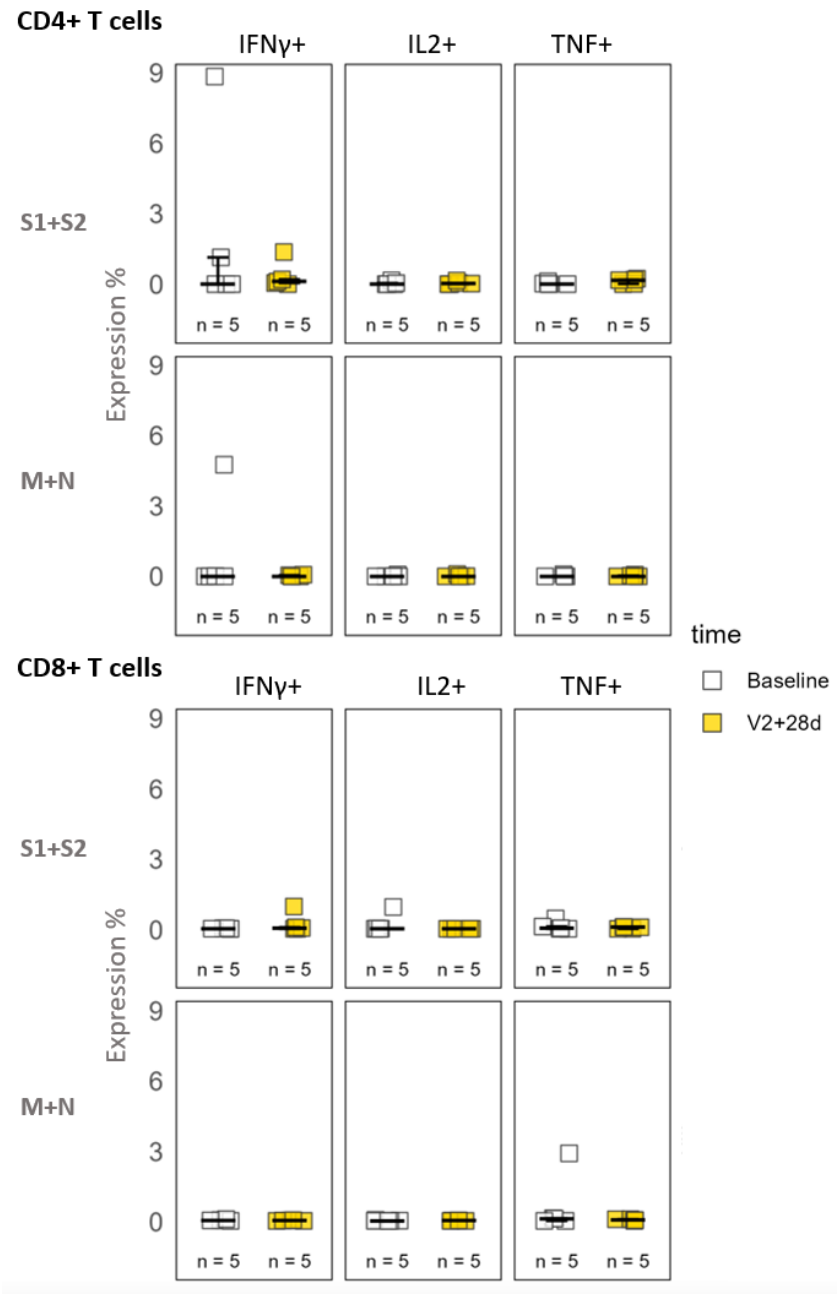
Supplementary Figure 13 Cytokine expression pre-V1 and post-V2 inactivated COVID-19 vaccine in SARS-CoV-2 naïve individuals

Peripheral blood mononuclear cells (PBMCs) from SARS-CoV-2 naïve individuals were collected pre-V1 and post-V2 of inactivated COVID-19 vaccines. Cells were stimulated for 16 hours with SARS-CoV-2 spike (S1+S2) and membrane plus nucleocapsid (MN) peptide pools. Naïve status was defined by seronegativity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons between pre- and post-vaccination timepoints were performed using the Wilcoxon signed-rank test. Statistical significance was defined as $p < 0.05$.



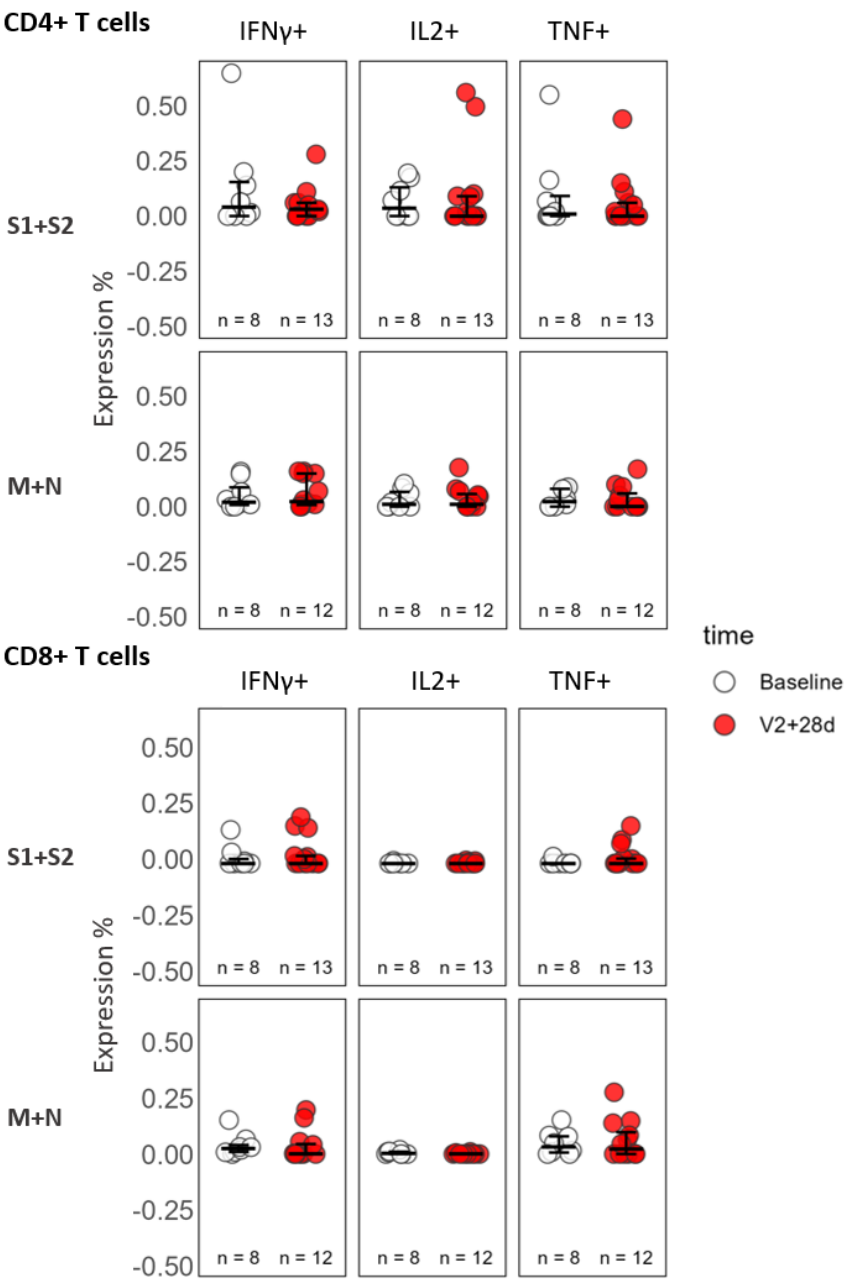
Supplementary Figure 14 Cytokine expression pre-V1 and post-V2 mRNA COVID-19 vaccine in SARS-CoV-2 naïve individuals

Peripheral blood mononuclear cells (PBMCs) from SARS-CoV-2 naïve individuals were collected pre-V1 and post-V2 of mRNA COVID-19 vaccines. Cells were stimulated for 16 hours with SARS-CoV-2 spike (S1+S2) and membrane plus nucleocapsid (MN) peptide pools. Naïve status was defined by seronegativity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN)-γ, interleukin (IL)-2 and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons between pre- and post-vaccination timepoints were performed using the Wilcoxon signed-rank test. Statistical significance was defined as $p < 0.05$.



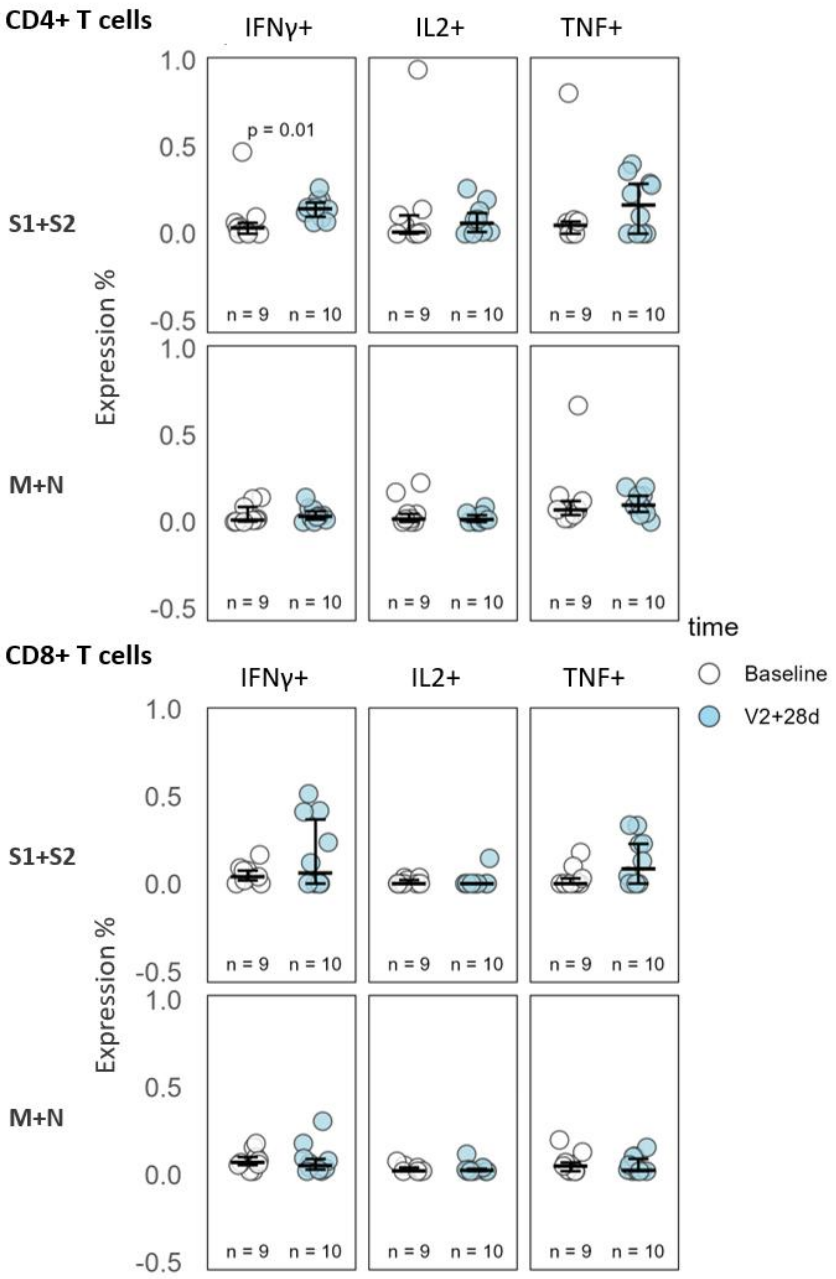
Supplementary Figure 15 Cytokine expression pre-V1 and post-V2 viral vector COVID-19 vaccine in SARS-CoV-2 naïve individuals

Peripheral blood mononuclear cells (PBMCs) from SARS-CoV-2 naïve individuals were collected pre-V1 and post-V2 of viral vector COVID-19 vaccines. Cells were stimulated for 16 hours with SARS-CoV-2 spike (S1+S2) and membrane plus nucleocapsid (MN) peptide pools. Naïve status was defined by seronegativity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons between pre- and post-vaccination timepoints were performed using the Wilcoxon signed-rank test. Statistical significance was defined as $p < 0.05$.



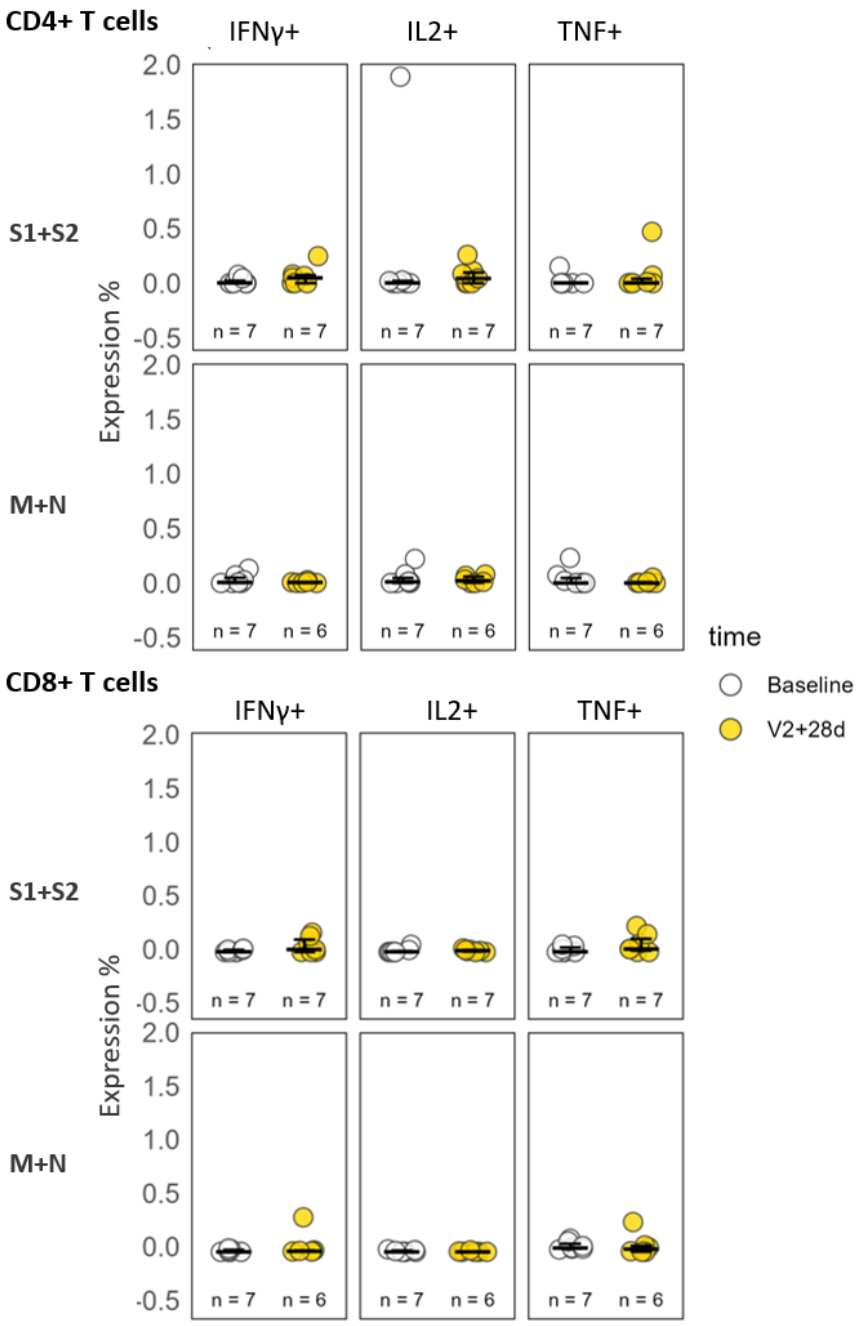
Supplementary Figure 16 Cytokine expression pre-V1 and post-V2 inactivated COVID-19 vaccine in SARS-CoV-2 previously infected individuals

Peripheral blood mononuclear cells (PBMCs) from SARS-CoV-2 previously infected individuals were collected pre-V1 and post-V2 of inactivated COVID-19 vaccines. Cells were stimulated for 16 hours with SARS-CoV-2 spike (S1+S2) and membrane plus nucleocapsid (MN) peptide pools. Previously infected status was defined by seropositivity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons between pre- and post-vaccination timepoints were performed using the Wilcoxon signed-rank test. Statistical significance was defined as $p<0.05$.



Supplementary Figure 17 Cytokine expression pre-V1 and post-V2 mRNA COVID-19 vaccine in SARS-CoV-2 previously infected individuals

Peripheral blood mononuclear cells (PBMCs) from SARS-CoV-2 previously infected individuals were collected pre-V1 and post-V2 of mRNA COVID-19 vaccines. Cells were stimulated for 16 hours with SARS-CoV-2 spike (S1+S2) and membrane plus nucleocapsid (MN) peptide pools. Previously infected status was defined by seropositivity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons between pre- and post-vaccination timepoints were performed using the Wilcoxon signed-rank test. Statistical significance was defined as $p<0.05$.



Supplementary Figure 18 Cytokine expression pre-V1 and post-V2 viral vector COVID-19 vaccine in SARS-CoV-2 previously infected individuals

Peripheral blood mononuclear cells (PBMCs) from SARS-CoV-2 previously infected individuals were collected pre-V1 and post-V2 of viral vector COVID-19 vaccines. Cells were stimulated for 16 hours with SARS-CoV-2 spike (S1+S2) and membrane plus nucleocapsid (MN) peptide pools. Previously infected status was defined by seropositivity to the nucleocapsid antigen at pre-V1. The proportion of CD4+ and CD8+ T-cells expressing interferon (IFN-)γ+, interleukin (IL)2+ and tumour necrosis factor (TNF) expressing cells was measured using intracellular cytokine staining (ICS). Errorbar represents the median and interquartile range (IQR) of cytokine expressing cells and individual datapoint are plotted. Comparisons between pre- and post-vaccination timepoints were performed using the Wilcoxon signed-rank test. Statistical significance was defined as $p<0.05$.

10.2 Supplementary tables

Supplementary Table 1 Research studies included in this thesis and associated ethical approval information

Study	Full title	Recruitment location	Ethics Ref
PITCH	Protective Immunity from T-cells to COVID-19 in Health workers	Oxford, Birmingham, Newcastle, Liverpool, Sheffield, Cambridge	REC ref.: 21/YH/0206
COCO	COVID-19 Convalescent immunity	Birmingham	REC ref.: 20/HRA/1817
COV-AD	COVID-19 in patients with antibody deficiency	Oxford, Birmingham, Newcastle, London, Leeds, Plymouth, Bristol, North Midlands	REC ref.: 21/LO/0162
SIREN	SARS-CoV-2 immunity and reinfection evaluation	UK wide	IRAS ID 284460, REC ref.: 20/SC/0230
VIBRANT	Vaccine immunity breakthrough & re-infection - antibody & T-cell	England & Scotland	REC ref.: 21/HRA/5433)
	Human immune responses to acute virus infections	Liverpool	REC ref. 16/NW/0170
STHObs	Observational Biobanking study	Sheffield	REC ref 18/YH/0441
EVAIC	Evaluation of adaptive immunity following inactivated whole-virus vaccination in Indonesian populations	Jakarta, Indonesia	KET-418/UN2.F1/ETIK/ PPM.00.02/2023 Oxford Tropical Research Ethics Committee (OxTREC) ref 544-23

Supplementary Table 2 List of antibodies used for CTV analysis

Marker Name	Fluorochrome	Clone	Species reactivity	Host species	Isotype	Manufacturer	Catalogue number	Dilution
Live/Dead	Near IR (APC-H7)							1:1000
CD3	FITC	UCHTI	Human	Mouse	IgG1, κ	Biolegend	300440	1:50
CD4	APC	RPA-T4	Human	Mouse	IgG1, κ	Biolegend	300514	1:200
CD8a	PE/Cyanine 7	RPA-T8	Human	Mouse	IgG1, κ	Biolegend	301012	1:200

Supplementary Table 3 77-marker immune memory and mechanisms of protection from vaccines (IMMPROVE) consortium panel

Description	Catalogue number	Company	Lot
Spark UV™ 387 anti-human CD8	344776	BioLegend	B415959
Spark UV™ 387 anti-human CD11b Antibody	301366	BioLegend	B392322
CD4 Monoclonal Antibody (SK3 (SK-3)), Brilliant Ultra Violet™ 395, eBioscience™	363-0047-41	Thermo	2745619
BD Horizon™ BUV395 Mouse Anti-Human CD4	*563550*	*BD*	*3334680*
BD Horizon™ BUV395 Mouse Anti-Human IgM	563903	BD	4019363
Mouse anti Human CD38:StarBright UltraViolet 445	MCA1019SBUV445	Bio-Rad	100003483
Mouse anti Human CD45RA:StarBright UltraViolet 510	MCA88SBUV510	Bio-Rad	100006379
Biotin anti-human CD57 Antibody	359616	BioLegend	B284173
Qdot™ 545 ITK™ Streptavidin Conjugate Kit	Q10091MP	Thermo	2344117
BD OptiBuild™ BUV563 Mouse Anti-Human CD127	748489	BD	4338647
BUV563 Mouse Anti-Human FCRL5 (CD307e) (bdbiosciences.com)	749598	BD	4142593
Ki-67 Monoclonal Antibody (SolA15), Brilliant Ultra Violet™ 615, eBioscience™	366-5698-82	Thermo	2961328
BD OptiBuild™ BUV661 Mouse Anti-Human CD278	741664	BD	4142528
BUV661 Mouse Anti-Human CD80 (bdbiosciences.com)	741647	BD	4142526
BUV737 Mouse Anti-Human CD161 (bdbiosciences.com)	748948	BD	4142586
BUV737 Mouse Anti-Human CD21 (bdbiosciences.com)	612789	BD	3194166
Rat anti Human CD28:StarBright UltraViolet 795	MCA709SBUV795	Bio-Rad	100006203
Mouse anti Human CD20:StarBright UltraViolet 795	MCA1710SBUV795	Bio-Rad	100006242

BD Horizon™ BUV805 Mouse Anti-Human CD16	569165	BD	4074596
Brilliant Violet 421™ anti-human Perforin Antibody	308122	BioLegend	B408076
Brilliant Violet 421™ anti-human CD40 Antibody	334332	BioLegend	B418048
Mouse anti Human CD19:StarBright Violet 440	MCA1940SBV440	Bio-Rad	100004784
Pacific Blue™ anti-human CD3 Antibody	300417	BioLegend	B394952
BD OptiBuild™ BV480 Mouse Anti-Human CD184 (CXCR4)	746621	BD	4142557
Mouse anti Human CD62L:StarBright Violet 515	MCA1076SBV515	Bio-Rad	100005057
Mouse anti Human CD27:StarBright Violet 570	MCA755SBV570	Bio-Rad	100005201
Brilliant Violet 605™ anti-human TCR Vα7.2 Antibody	351720	BioLegend	B430579
Brilliant Violet 605™ anti-human CD1c Antibody	331538	BioLegend	B374696
TCRγ/δ Antibody, anti-human, REAfinity™	130-122-291	Miltenyi	5241002807
BD OptiBuild™ BV650 Mouse Anti-Human IgG	740596	BD	4257682
Brilliant Violet™ 711 anti-human CD152 (CTLA-4) Antibody	369632	BioLegend	B378192
BV711 Mouse Anti-Human CD268 (BAFF Receptor) (bdbiosciences.com)	743573	BD	4142542
Brilliant Violet 750™ anti-human CD223 (LAG-3) Antibody	369352	BioLegend	B399990
Brilliant Violet 750™ anti-human CD10 Antibody	312244	BioLegend	B417426
Brilliant Violet 785™ anti-human CD183 (CXCR3) Antibody	353738	BioLegend	B384611
BD Horizon™ BB515 Mouse Anti-Human CD40L (CD154)	568170		4019504
BD Horizon™ BB515 Streptavidin	564453	BD	3212683
KIRAVIA Blue 520™ anti-human CD197 (CCR7)	353259	BioLegend	B373660
BD OptiBuild™ RB545 Mouse Anti-Human TCR Vα24	758082	BD	4142614
BD OptiBuild™ RB545 Mouse Anti-Human Vγ9 TCR	756541	BD	4338652
BD Horizon™ RB545 Mouse Anti-Human CD24	569746	BD	3150011
Spark Blue™ 574 anti-human HLA-DR, DP, DQ	361724	BioLegend	B414145
BD Horizon™ RB613 Mouse Anti-Human CD279 (PD-1)	571093	BD	4002456
Mouse anti Human CD69:StarBright Blue 615	MCA2806SBB615	Bio-Rad	100006839
BB660-P2 Mouse Anti-Human CD39 Clone TU66 (50ug)	Custom	BD	5051505
Streptavidin Brilliant Blue 660-P2	Custom	BD	4162116
PerCP anti-human CD14 Antibody	367152	BioLegend	B431715
BD Horizon™ BB700 Mouse Anti-Human CD25	567482	BD	4032515
BD Horizon™ BB700 Mouse Anti-Human CD25 IL-2 Receptor α chain, p55	566448	BD	4015809

BD OptiBuild™ BB700 Mouse Anti-Human CD141 Thrombomodulin	742245	BD	4180656
RB705 Mouse Anti-TCF7 (TCF1) (bdbiosciences.com)	570635	BD	4183971
RB705 Mouse Anti-Human CD138 (bdbiosciences.com)	756962	BD	4142612
BD OptiBuild™ RB744 Rat Anti-Human CXCR5 (CD185)	757113	BD	4156872
RB780 Mouse Anti-Bcl-6 (bdbiosciences.com)	569143	BD	3320214
PerCP/Fire™ 806 anti-human KLRG1 (MAFA)	367747	BioLegend	B408827
PerCP/Fire™ 806 anti-human IgD	307817	BioLegend	B392544
PE anti-human CD134 (OX40)	350003	BioLegend	B331568
PE Streptavidin	405204	BioLegend	NA
IgE Monoclonal Antibody (Ige21), PE, eBioscience™	*12-6986-42*	*Thermo*	2732783
Spark YG™ 593 anti-human CD71 Antibody	334144	BioLegend	B416246
Gata-3 Monoclonal Antibody (TWAJ), PE-eFluor™ 610, eBioscience™	61-9966-42	Thermo	2892934
CD23 Monoclonal Antibody (EBVCS2), PE-eFluor™ 610, eBioscience™	61-0238-42	Thermo	3030617
PE/Fire™ 640 anti-human CD11c	337246	BioLegend	B411144
PE/Cyanine5 anti-human CD335 (NKp46) Antibody	331952	BioLegend	B416991
PE/Cyanine5 anti-human CD86	305408	BioLegend	B379759
PE/Fire™ 700 anti-human CD194 (CCR4)	359436	BioLegend	B418621
PE/Fire™ 744 anti-human CD137 (4-1BB)	309851	BioLegend	B414905
BD Horizon™ RY775 Mouse Anti-Human Granzyme B	571392	BD	4100433
PE/Fire™ 810 anti-human TIGIT (VSTM3) Antibody	372745	BioLegend	B409947
APC anti-human CD49a Antibody	328314	BioLegend	B402846
APC Streptavidin	405207	BioLegend	NA
IgA Antibody, anti-human, APC	130-113-472	Miltenyi	5241000013
Alexa Fluor® 647 anti-human FOXP3	320114	BioLegend	B369015
Spark NIR™ 685 anti-human CD56 (NCAM) Antibody	362563	BioLegend	B406084
BD Horizon™ R718 Mouse Anti-T-bet	567096	BD	4151430
BD OptiBuild™ R718 Mouse Anti-Human CD123	752032	BD	4257669
ViaKrome 808 Fixable Viability Dye	C36628	Beckman Coulter	200049
APC/Fire™ 750 anti-human CD95 (Fas) Antibody	305638	BioLegend	B379296
APC/Fire™ 810 anti-human CD196 (CCR6) Antibody	353451	BioLegend	B412352

Supplementary Table 4 Surface master mix reagents and dilution

Fluorophore	Marker	Dilution
Buffer	Cell staining buffer	
Blocking	Brilliant Stain	3.3
Blocking	Tandem Stabilizer	1000
Blocking	Biotin (500uM stock)	1000
RB744	CXCR5	1000
BUV563	FCRL5	500
BV650	TCRgd (REA pure)	200
BB700	CD25 (MA251)	200
PerCP-Fire 806	IgD	200
PE-Fire 700	CCR4	200
BUV661	CD80	100
SBUV700	CD62L	100
BV480	CXCR4	100
SBV535	CD27	100
BV785	CXCR3	100
Biotin	CD57	100
BB700	CD25 (BC96)	100
APC-Fire 810	CCR6	100
BUV563	CD127	50
SBV515	CD103	50
PE-Fire 810	TIGIT	50
APC-Fire 750	CD95	50
SBV570	CCR5	20
KB520	CCR7	20
PE	IgE	200
BV421	CD40	50
ViaKrome 808	Viability	300
Second step:		
Buffer	Cell staining buffer	
Blocking	Tandem Stabilizer	1000
SBUV540	Streptavidin	200

Supplementary Table 5 Blocking mix reagents and dilution for immunophenotyping

Blocking mix	Catalogue Number	Dilution	Company
Mouse serum	10410	3.3	Thermo Scientific
Rat serum	10710C	3.3	Thermo Scientific
FACS buffer		9.4	
Tandem Stabilizer	421802	1000	BioLegend
Sodium Azide (10% solution)		100	

Supplementary Table 6 Intracellular master mix reagents and dilution

Fluorophore	Marker	Dilution
Buffer	10x Perm/Wash buffer	10
Buffer	Cell staining buffer	
Blocking	Brilliant Stain (00-4409-75, Thermo)	3.3
Blocking	Tandem Stabilizer	1000
Pacific Blue	CD3	1000
PerCP-Fire 806	KLRG1	1000
BV650	IgG	500
BV711	BAFFR	500
RY775	GranzymeB	500
R718	CD123	500
Spark Blue 574	HLADR	250
BUV395	CD4 (Thermo)	200
BUV661	ICOS	200
BV605	CD1c	200
BB700	CD141	200
RB705	CD138	200
PE-Cy5	CD86	200
Spark YG 593	CD71	200
PE-eFluor610	CD23	200
Spark UV 387	CD8	100
Spark UV 387	CD11b	100
BUV615	Ki67	50
BUV737	CD161	100
SBUV770	CD20	100
SBUV770	CD28	100
BUV805	CD16	100
SBV440	CD19	100
BV711	CTLA4	100
BV750	CD10	100
BV750	LAG3	100
RB545	TCRVa24	100
RB545	CD24	100
RB613	PD1	100
PerCP	CD14	100
RB705	TCF1	100
RB780	Bcl6	100
PE-Fire 640	CD11c	100
PE-Cy5	NKp46	100
PE-Fire 744	CD137	100
R718	Tbet	100
BUV395	IgM	50
SBUV445	CD38	50
SBUV510	CD45RA	50
BUV737	CD21	50

SBV515	CD163	50
BV605	TCRVa72	50
AF647	FoxP3 (206D)	50
SBB615	CD69	40
RB545	TCRVg9	25
PE-eFluor610	GATA3	25
Spark NIR 685	CD56	20
BB660	CD39	200
BB515	CD154	2000
APC	CD49a	200
APC	IgA	2000
PE	CD134	200
BV421	Perforin	100

Supplementary Table 7 List of antibodies used for extracellular staining flow cytometry

Marker Name	Fluorochrome	Clone	Species reactivity	Host species	Isotype	Manufacturer	Catalogue number	Dilution
Live/Dead	Near IR (APC-H7)					Invitrogen	L34976	1:1000
CD14	APC-Fire750	M5E2	Human	Mouse	IgG2a	Biolegend	301854	1:200
CD3	BV510	UCHTI	Human	Mouse	IgG1, κ	Biolegend	300448	1:100
CD4	APC	RPA-T4	Human	Mouse	IgG1, κ	Biolegend	300514	1:200
CD8a	PE-Vio770	BW135/80	Human	Mouse	IgG2a, k	Miltenyi	130-098-060	1:100
CD56	BV421	HCD56	Human	Mouse	IgG1, κ	Biolegend	318327	1:50
CD69	PE	FN50	Human	Mouse	IgG1, κ	Biolegend	310906	1:50
HLA-DR	FITC	L243	Human	Mouse	IgG2a, κ	eBioscience	307604	1:30

Supplementary Table 8 List of antibodies used for intracellular staining flow cytometry

Marker Name	Fluorochrome	Clone	Species reactivity	Host species	Isotype	Manufacturer	Catalogue number	Dilution
Live/Dead	Near IR (APC-H7)					Invitrogen	L34976	1:1000
CD14	APC-Fire750	M5E2	Human	Mouse	IgG2a	Biolegend	301854	1:200
CD3	PerCP	UCHT1	Human	Mouse	IgG1, κ	Biolegend	300440	1:100
CD4	FITC	RPA-T4	Human	Mouse	IgG1, κ	Biolegend	300506	1:100
CD8a	BV510	RPA-T8	Human	Mouse	IgG1, κ	Biolegend	301048	1:600
CD56	BV421	HCD56	Human	Mouse	IgG1, κ	Biolegend	318328	1:50
IFN-γ	PE	4S.B3	Human	Mouse	IgG1, κ	Biolegend	502509	1:50
IL2	PE/Cyanine7	MQ1-17H12	Human	Rat	IgG2a, κ	eBioscience	25-7029-42	1:160
TNFα	APC	Mab11	Human	Mouse	IgG1	Biolegend	502912	1:100

Supplementary Table 9 Healthcare worker role classification by patient-facing

Patient-facing role	Non-patient-facing role
Chaplain	Administration
Clinical Psychologist	Biomedical Scientist
Consultant	Clinical Support Assistant
Doctor	Healthcare Scientist
Junior Doctor	Manager
Midwife	Medical Student
Nursing	Laboratory Staff
Physiotherapist	Pharmacist
Radiographer	Rehabilitation Engineer
Registered Nurse	Research Support Specialist

Supplementary Table 10 Differences of replication and transcription complex immunogenic peptides used between Swadling *et al*. and this thesis

	Protein (amino acid residues)	Swadling *et al*. (SARS-CoV-2 amino acid sequence) (Swadling et al., 2022)	NKI study (SARS-CoV-2 amino acid sequence)
CD4	NSP7 (21-35)	RVESSSKLWAQCVQL	LRVESSSKLWAQCVQL
	NSP7 (51-65)	KMVSLLSVLLSMQGA	EKMVSLLSVLLSMQGAV
	NSP7 (71-85)	NKLCEEMLDNRATLQ	KLCEEMLDNRATLQAI
	NSP12 (436-450)	ELKHFFFAQDGNAAI	LKHFFFAQDGNAAISDY
	NSP12 (446-460)	GNAAISDYDYYRYNL	QDGNAAISDYDYYRYNL
	NSP12 (851-865)	DGTLMIERFVSLAID	IVKTDGTLMIERFVSLAI
	NSP13 (391-405)	LRAKHYVYIGDPAQL	RLRAKHYVYIGDPAQL
	NSP13 (586-600)	FTSLEIPRRNVATLQ	DKLQFTSLEIPRRNVATL
CD8	NSP7 (21-35)	RVESSSKLWAQCVQL	LRVESSSKLWAQCVQL
	NSP12 (436-450)	ELKHFFFAQDGNAAI	LKHFFFAQDGNAAISDY
	NSP12 (446-460)	GNAAISDYDYYRYNL	QDGNAAISDYDYYRYNL
	NSP12 (826-840)	YVYLPYPDPSRILGA	LVKQGDDYVYLPYPDPSR
	NSP13 (391-405)	LRAKHYVYIGDPAQL	RLRAKHYVYIGDPAQL
	NSP13 (586-600)	FTSLEIPRRNVATLQ	DKLQFTSLEIPRRNVATL

Supplementary Table 11 Proteins with statistically significant differences in expression between serum and plasma

Protein	log2 fold change	W statistic	W p-value	W adj. p-value	Correlation	Corr. p-value
ANGPT1	3.494	528	0.000	0.000	0.162	0.376
ARHGEF12	1.746	424	0.003	0.006	-0.120	0.513
AXIN1	1.245	407	0.008	0.014	0.019	0.916
BACH1	1.318	459	0.000	0.001	0.303	0.092
CASP2	2.540	484	0.000	0.000	0.055	0.765
CCL17	2.399	520	0.000	0.000	0.304	0.091
CCL26	-1.179	141	0.022	0.036	-0.045	0.805
CCL3	2.357	527	0.000	0.000	0.353	0.048
CCL4	2.055	528	0.000	0.000	0.478	0.006
CCN2	2.251	528	0.000	0.000	0.266	0.141
CD40LG	3.345	528	0.000	0.000	0.306	0.088
CD84	1.316	526	0.000	0.000	0.264	0.144
CLEC4D	1.689	523	0.000	0.000	0.726	0.000
CRKL	2.057	449	0.001	0.002	0.001	0.997
CST7	1.814	515	0.000	0.000	0.383	0.030
CXCL1	2.148	505	0.000	0.000	0.077	0.677
CXCL17	-1.036	12	0.000	0.000	0.712	0.000
CXCL3	2.403	480	0.000	0.000	0.163	0.372
CXCL6	1.626	485	0.000	0.000	-0.053	0.773
CXCL8	2.944	516	0.000	0.000	-0.109	0.554
DAG1	1.879	520	0.000	0.000	0.236	0.193
DBNL	2.577	468	0.000	0.000	-0.011	0.954
DNAJA2	2.495	483	0.000	0.000	-0.009	0.960
EDAR	2.684	517	0.000	0.000	0.262	0.147
EGF	4.896	526	0.000	0.000	-0.038	0.837
EGLN1	1.871	473	0.000	0.000	0.010	0.959
F2R	-2.561	7	0.000	0.000	0.432	0.014
FKBP1B	3.139	475	0.000	0.000	-0.086	0.641
GMPR	-1.734	22	0.000	0.000	0.481	0.005
HCLS1	1.225	463	0.000	0.001	0.168	0.357
HGF	1.494	528	0.000	0.000	0.617	0.000
ICA1	1.522	451	0.000	0.001	0.067	0.717
IKBKG	1.408	408	0.007	0.013	0.037	0.841
IL1B	1.264	436	0.001	0.003	0.021	0.908
IL4	1.900	419	0.004	0.008	0.171	0.350
IL6	1.009	444	0.001	0.002	0.411	0.020
IL7	2.670	528	0.000	0.000	0.404	0.022
IRAK1	1.669	465	0.000	0.001	0.005	0.976
LAMA4	1.181	510	0.000	0.000	0.143	0.435
LGMN	1.427	528	0.000	0.000	0.175	0.337
LSP1	1.850	522	0.000	0.000	0.282	0.118
MANF	2.148	442	0.001	0.002	-0.214	0.239

MAP2K6	2.870	482	0.000	0.000	-0.068	0.712
MMP1	1.967	528	0.000	0.000	0.618	0.000
MPIG6B	3.541	523	0.000	0.000	-0.162	0.376
MYO9B	1.185	427	0.002	0.005	-0.164	0.370
NBN	2.090	473	0.000	0.000	-0.018	0.921
NCF2	3.132	509	0.000	0.000	0.081	0.659
NFATC1	1.576	446	0.001	0.002	-0.009	0.960
OSM	2.268	522	0.000	0.000	0.438	0.012
PDGFB	2.969	525	0.000	0.000	0.230	0.205
PDLIM7	2.986	470	0.000	0.000	0.052	0.776
PIK3AP1	1.353	455	0.000	0.001	0.088	0.634
PLAUR	1.192	520	0.000	0.000	0.396	0.025
PLXNA4	1.112	413	0.005	0.011	0.212	0.245
PPP1R9B	1.108	396	0.014	0.024	0.176	0.335
PRDX5	1.925	509	0.000	0.000	0.365	0.040
PRKAB1	1.780	478	0.000	0.000	-0.155	0.398
PSIP1	1.020	439	0.001	0.003	-0.092	0.618
RAB6A	2.210	510	0.000	0.000	0.342	0.055
SAMD9L	1.839	449	0.001	0.002	0.018	0.921
SKAP2	3.008	485	0.000	0.000	0.036	0.845
STX8	1.424	436	0.001	0.003	0.125	0.494
TBC1D5	1.634	411	0.006	0.012	-0.160	0.381
TGFA	2.079	528	0.000	0.000	0.349	0.051
TGFB1	1.131	526	0.000	0.000	0.538	0.002
TIMP3	3.547	525	0.000	0.000	0.114	0.534
TNFRSF14	1.559	525	0.000	0.000	0.364	0.041
TNFSF12	1.088	528	0.000	0.000	0.421	0.016
TPP1	1.583	528	0.000	0.000	0.162	0.375
TRIM5	1.294	411	0.006	0.012	-0.015	0.935
VEGFA	1.393	528	0.000	0.000	0.408	0.020
WAS	2.247	469	0.000	0.000	-0.024	0.897

Supplementary Table 12 Proteins with statistically significant differences in expression (without correction due to multiple testing) between T-cell response and non-response groups

Protein	log2 fold change	W statistic	W p-value	W adj. p-value
CCL13	-0.241	1251.0	0.0153	0.674
CCL26	-0.385	1290.0	0.0256	0.674
CCL7	-0.311	1315.0	0.0349	0.716
CD40	-0.458	1113.0	0.0019	0.192
COL9A1	0.691	2369.0	0.0021	0.192
EDAR	-0.679	1326.0	0.0399	0.716
EIF4G1	-1.122	1090.0	0.0013	0.192
FABP9	0.206	2173.0	0.0349	0.716
FGF5	0.629	2165.0	0.0385	0.716
FIS1	-0.533	1331.0	0.0423	0.716
IL18R1	-0.220	1343.5	0.0490	0.716
IL2RB	0.618	2163.0	0.0394	0.716
IL5RA	0.329	2151.0	0.0454	0.716
ITGB6	-0.204	1283.0	0.0234	0.674
ITM2A	0.726	2264.0	0.0106	0.674
LGALS9	-0.185	1286.5	0.0245	0.674
LTA	0.404	2197.0	0.0259	0.674
MANF	-0.875	1271.0	0.0200	0.674
METAP1D	-0.758	1232.5	0.0119	0.674
PLXNA4	-0.614	1319.0	0.0367	0.716
PRDX3	-0.561	1344.0	0.0493	0.716
PRSS8	-0.372	1000.0	0.0003	0.092
SCG3	0.773	2216.0	0.0203	0.674
TPSAB1	0.686	2230.5	0.0167	0.674

Supplementary Table 13 Odds ratios of T-cell responsiveness predictors following two doses of BNT162b2 COVID-19 vaccine

	T_responder		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	36.75046	36.66271 – 36.83842	**<0.001**
Age	1.01533	1.01291 – 1.01776	**<0.001**
Sex [M]	0.07925	0.07906 – 0.07944	**<0.001**
EIF5A	2.75775	2.75117 – 2.76435	**<0.001**
IL1RL2	0.46560	0.46448 – 0.46671	**<0.001**
COL9A1	3.26173	3.25394 – 3.26953	**<0.001**
MMP1	0.60029	0.59885 – 0.60172	**<0.001**
SKAP2	0.87087	0.86879 – 0.87295	**<0.001**
IgG CMV [1]	0.53629	0.53501 – 0.53757	**<0.001**
Random Effects			
σ^2	3.29		
$\tau_{00\ Participant.ID}$	20.41		
$\tau_{00\ type}$	0.00		
$\tau_{00\ visit\_cat}$	0.00		
ICC	0.86		
N_{visit_cat}	2		
$N_{Participant.ID}$	117		
N_{type}	3		
Observations	137		
Marginal R^2 / Conditional R^2	0.180 / 0.886		

Supplementary Table 14 Demographic and clinical characteristics of cohorts included in the immunophenotyping analysis

	Healthy		Immunocompromised patients	
Characteristics	**T-cell non-response group** (n=23)	**T-cell response group** (n=18)	**T-cell non-response group (n=7)**	**T-cell response group** (n=11)
Age, year (range, mean ± SD)	26-56, 43.7±9.2	22-48, 28.2±7.7	30-70, 46.9±17.2	32-58, 44.5±9.1
Sex, n (%)				
Male	8 (34.8%)	7 (38.9%)	2 (28.6%)	4 (36.4%)
Female	15 (65.2%)	11 (61.1%)	5 (71.4%)	7 (63.6%)
History of COVID-19	0	0	0	1 (5.6%)
Diagnosis				
Undefined combined immunodeficiency	NA	NA	1 (14.3%)	-
Common variable immunodeficiency disorder	NA	NA	1 (14.3%)	9 (81.8%)
Hypogammaglobulinaemia	NA	NA	-	1 (9.1%)
Other primary antibody deficiencies	NA	NA	-	1 (9.1%)
Secondary - Haematological cause	NA	NA	2 (28.6%)	-
Secondary - Rheumatological cause	NA	NA	1 (14.3%)	-
Specific polysaccharide antibody deficiency	NA	NA	2 (28.6%)	-

10.3 Case studies of the two individuals without any immune indicators of SARS-CoV-2 infection

Participant A, a HCW with a patient-facing role, had additional samples collected and available at V4+11 months (blood and nasal swab - September 2023), V5+1 month (blood - October 2023) or V5+2 months (nasal swab-November 2023), corresponding to 8, 9 and 10 months after the measured timepoint in this study (V4+3 month, January 2023). Participant A had S-specific antibodies but remained seronegative to SARS-CoV-2 N antibody in both nasal swab and blood (Figure 10-1a-c). Participant A showed a two-fold increase (130 SFU/10^6 PBMCs) in IFN-γ-secreting cell response against spike 7 months since their previous visit (60 SFU/10^6 PBMCs) despite no additional vaccine, and then a further increase (195 SFU/10^6 PBMCs) one month after their fifth vaccine dose (Figure 10-2a, Table 10-1). The participant also had IFN-γ-secreting cells against non-spike proteins above the cut-off for detection (30 SFC/10^6 PBMC) at both timepoints.

Participant B, a HCW with a non-patient-facing role, had one additional sample collected at V3+12 months (blood and nasal swab - November 2022), 5 months after the measured timepoint in our study, V3+7 months (blood and nasal swab - June 2022). Participant B was lost to follow up after November 2022, so no further information following that visit. Participant B had antibodies to S but became negative for nasal IgA at V3+12 months (Figure 10-1d-f). Participant B remained negative for nucleocapsid-specific antibodies. Participant B showed an increase in IFN-γ-secreting cells to spike, despite no additional vaccine dose, but continued to have no detectable responses to non-spike proteins (Figure 10-2b).

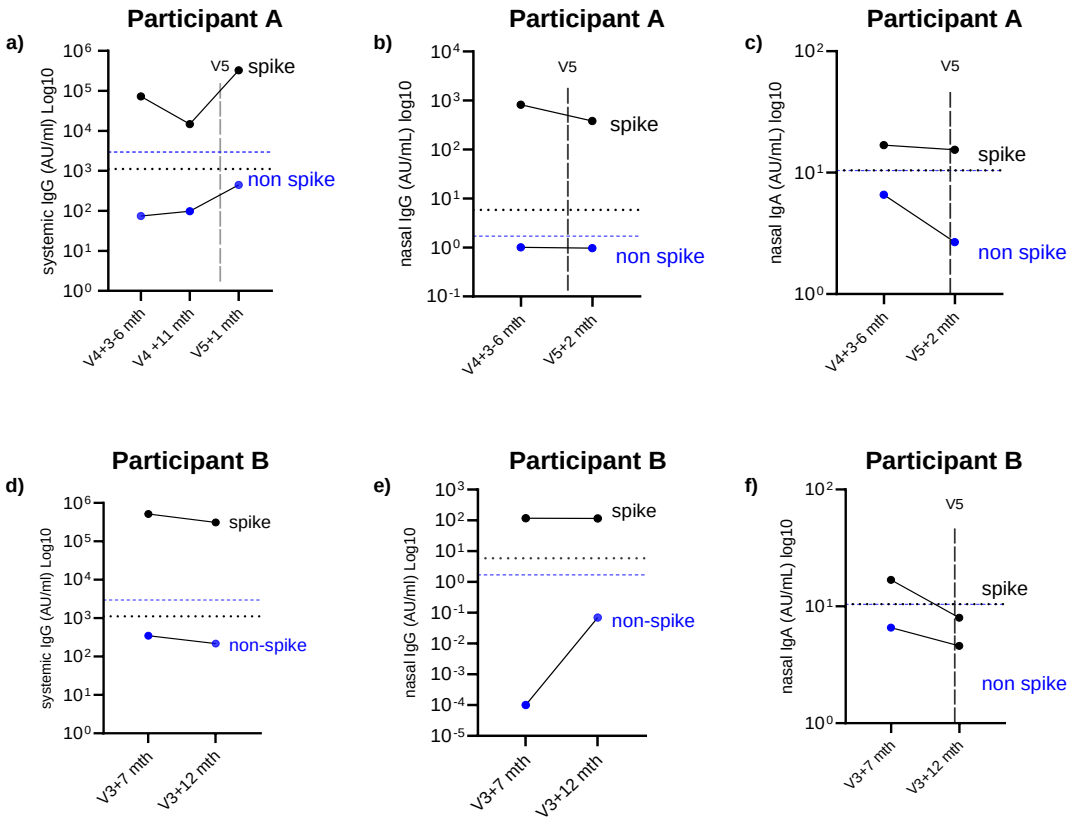


Figure 10-1 Longitudinal analysis of antibody responses in participants with initially negative assay results

Participants A (a, b, c) and B (d, e, f) who were negative across all immune assays were further analysed using longitudinal samples to assess whether their antibody responses changed over time. a), d) Systemic antibodies, b) e) nasal IgG and c), f) nasal IgA were measured against SARS-CoV-2 spike and nucleocapsid (non-spike). The black dotted line indicates the positive cut-off for spike, while the blue dashed line for non-spike.

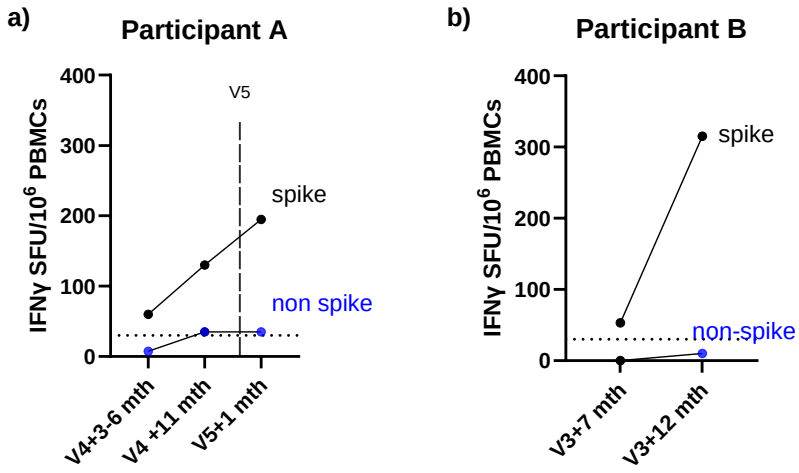


Figure 10-2 Longitudinal analysis of ELISpot T-cell responses in participants with initially negative assay results

Participants who were negative across all immune assays were further analysed using longitudinal samples to assess whether their T-cell responses changed over time. PBMCs were stimulated for 16-18 hours with overlapping SARS-CoV-2 peptides, including spike (S), membrane and nucleocapsid proteins (M+N), non-structural proteins (NSP3, 7-11, 12, 13), and open reading frame (ORF)3. IFN-γ spot forming unit (SFU) per 1 million cells are shown. The cut-off was set at the mean background + 2SD (dashed line). V5 is the timepoint of the fifth vaccine dose.

Participant A had no detectable proliferative T-cells at V4+3-6 months (Figure 10-3a,b), but had proliferative $CD8^+$ T-cells against NSP7-11 at V4+11 months (Table 10-1). Following their fifth dose, participant A had CD4+ T-cells to S, NSP12b and NSP13 at V5+1 month (Figure 10-3a, Table 10-1). Participant B had $CD4^+$ T-cells to S2 at V3+7 months but did not have $CD4^+$ and $CD8^+$ T-cell proliferative responses to spike and non-spike proteins one year after their third dose (Figure 10-3c,d, Table 10-1).

Overall, my finding showed that Participant A and B had robust antibody and T-cell responses to SARS-CoV-2 S between June 2022 and November 2023, when Omicron variant was circulating in the population. Participant B remained to be negative for immune responses to non-spike, while Participant A had non-spike immune responses, suggesting an infection at V4+11 months.

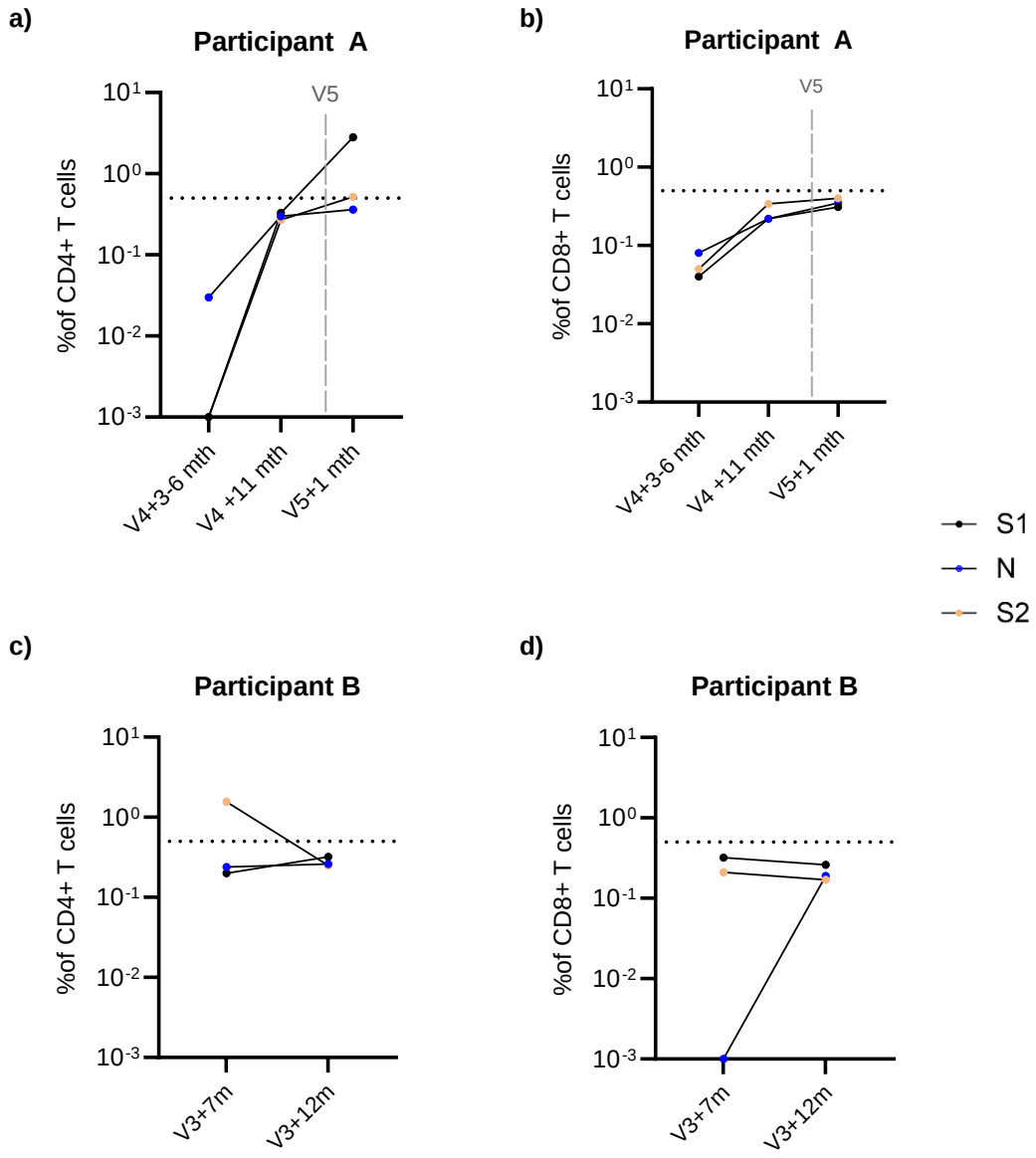


Figure 10-3 Longitudinal analysis of proliferative T-cell responses in participants with initially negative assay results

Participants who were negative across all immune assays were further analysed using longitudinal samples to assess whether their T-cell responses changed over time. PBMCs were stimulated for 7 days with overlapping SARS-CoV-2 peptides, including spike (S)1, S2 and nucleocapsid (N) proteins. The proliferation percentage of (a, c) CD4+ T-cells and (b, d) CD8+ T-cells are plotted after subtracted to responses detected in the background well. Values of zero are displayed as 10^{-3} for graphical representation. The cut-off was set at 0.5% (dashed line). V5 is the timepoint of the fifth vaccine dose.

Table 10-1 Results of SARS-CoV-2 non-spike-specific immune responses of longitudinal samples in participants who were previously had no marker of SARS-CoV-2 infection

		Participant A			Participant B	
	Positive cut-off	**V4+3-6 month**	**V4+11 month**	**V5+1/2 month***	**V3+7 month**	**V3+12 month**
Antibody (AU/mL)						
Systemic IgG to S	1120.59	72,402	14,729	325,861	516,057	310,895
Systemic IgG to N	2957.25	74.49	97.91	443.22	349.43	217.60
Nasal IgG to S	1.70	821.93	116.43	380.20	117.64	116.43
Nasal IgG to N	5.87	1.01	NA	0.97	101.28	0.07
Nasal IgA to S	505.37	16.84	8.00	15.47	16.84	8.00
Nasal IgA to N	1358.69	6.59	NA	2.68	160.59	4.59
IFN-γ-secreting cells (SFU 10^6 PBMCs)						
T-cell to spike	30	60	130	195	53	315
T-cell to non-spike	30	7.5	35	35	0	10
Proliferative T-cell responses (%)						
CD4+ T-cells to S1	0.5	0.001	0.33	2.81	0.2	0.32
CD4+ T-cells to S2	0.5	0.001	0.27	0.52	1.56	0.25
CD4+ T-cells to N	0.5	0.03	0.3	0.36	0.24	0.26
CD4+ T-cells to M	0.5	0	0.26	0.35	0	0.15
CD4+ T-cells to NSP3b	0.5	0.09	0.28	0.29	0.28	0.17
CD4+ T-cells to NSP 7-11	0.5	0.03	0.3	0.27	0.25	0.14
CD4+ T-cells to NSP12a	0.5	0.18	0.25	0.43	0.31	0.25
CD4+ T-cells to NSP12b	0.5	0.23	0.4	0.61	0.02	0.25

CD4+ T-cells to NSP 13	0.5	0.05	0.41	0.55	0.1	0.23
CD4+ T-cells to ORF3	0.5	0.01	0.32	0.28	0	0.17
CD8+ T-cells to S1	0.5	0.04	0.22	0.31	0.32	0.26
CD8+ T-cells to S2	0.5	0.05	0.34	0.40	0.21	0.17
CD8+ T-cells to N	0.5	0.08	0.22	0.35	0	0.19
CD8+ T-cells to M	0.5	0.01	0.32	0.25	0.29	0.23
CD8+ T-cells to NSP3b	0.5	0.06	0.31	0.25	0.11	0.21
CD8+ T-cells to NSP 7-11	0.5	0	0.57	0.23	0.07	0.12
CD8+ T-cells to NSP12a	0.5	0.06	0.3	0.32	0.04	0.3
CD8+ T-cells to NSP12b	0.5	0.1	0.28	0.43	0	0.21
CD8+ T-cells to NSP 13	0.5	0	0.39	0.33	0	0.24
CD8+ T-cells to ORF3	0.5	0	0.27	0.27	0	0.25

*Participant A had plasma sample collected at V5+1 month, but their nasal swab was collected at V5+2 months