Die Wirksamkeit von mRNA-Impfstoffen bei Beschäftigten des Universitätsklinikums Bonn und die Inzidenz von SARS-CoV-2-Infektionen

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To all those who were tireless in their efforts to save lives during the pandemic, and those whose lives could not be saved.

An alle, die während der Pandemie unermüdlich daran gearbeitet haben, Leben zu retten, und an all diejenigen, die ihr Leben verloren haben.

Inhaltsverzeichnis

Abkürzungsverzeichnis

ACE-2-Rezeptor	Angiotensin-konvertierendes Enzym 2-Rezeptor
AfAMed	Ausschuss für Arbeitsmedizin
AMR	Arbeitsmedizinische Regel
ArbSchG	Arbeitsschutzgesetz
ArbMedVV	Verordnung zur arbeitsmedizinischen Vorsorge
ASiG	Arbeitssicherheitsgesetz
BAU	binding antibody units (Antigen bindende Einheiten)
BAuA	Bundesanstalt für Arbeitsschutz und Arbeitsmedizin
BMG	Bundesministerium für Gesundheit
CoronaSchVO	Coronaschutzverordnung
COVID-19	Coronavirus-Krankheit-2019
ELISA	Enzyme-linked Immunosorbent Assay (Enzymgekoppelter Immunosorbent-Test)
EMA	European Medicines Agency (Europäische Arzneimittelbehörde)
FACS	fluorescence activated cell sorting (fluoreszenzaktivierte Zellsortierung)
IC_{50}	Mittlere inhibitorische Konzentration
IfSG	Infektionsschutzgesetz
IgA-AK	Immunglobulin A-Antikörper
IgG-AK	Immunglobulin G-Antikörper
INZ	Interdisziplinäres Notfallzentrum
KI	Kumulative Inzidenz
KITA	Kindertagesstätte
mRNA	messenger ribonucleic acid (Boten-Ribonukleinsäure)
OAS	original antigenic sin (Antigenerbsünde)
PBMC	peripheral blood mononuclear cell (periphere mononukleäre Blutzelle)
RT-PCR	Reverse-Transkriptase-Polymerase-Kettenreaktion

RKI	Robert Koch-Institut
SARS-CoV-2	Schweres-akutes-Atemwegssyndrom-Coronavirus Typ 2
STIKO	Ständige Impfkommission
TRBA	Technische Regeln für Biologische Arbeitsstoffe
UKB	Universitätsklinikum Bonn
VOC	variants of concern (besorgniserregende Varianten)
WE	Willkürliche Einheit
WT	Wild-Typ-Virusvariante

1. Deutsche Zusammenfassung

1.1 Einleitung

Im Dezember 2019 wurde erstmals das neuartige Coronavirus SARS-CoV-2 (Schweres-akutes-Atemwegssyndrom-Coronavirus Typ 2), Erreger der Erkrankung COVID-19 (Coronavirus disease 2019), in China identifiziert (Huang et al., 2020, Hu et al., 2021). Es folgte die globale Verbreitung des Virus, sodass am 11. März 2020 die Weltgesundheitsorganisation (WHO) den Pandemiefall feststellte (Ghebreyesus, 2020).

In der Bundesrepublik Deutschland wurden die ersten beiden Todesfälle durch das neuartige Coronavirus (SARS-CoV-2) am 09. März 2020 gemeldet (Böhmer et al., 2020). Bis zum 17. März 2023 stieg die Zahl der Todesfälle in Zusammenhang mit dem Virus auf 169.579 (Robert Koch-Institut, Neuartiges Coronas-Virus Situationsbericht vom 17.03.2023). Bis zu diesem Tag wurden dem Robert Koch-Institut (RKI) bundesweit mehr als 38,2 Millionen Infektionen gemeldet. Mit Stand vom 19.10.2023 sind seit Beginn der COVID-19 Impfkampagne weltweit mehr als 13.516.282.548 COVID-19-Impfdosen verabreicht worden und die kumulative Zahl der weltweit bestätigten SARS-CoV-2-Infektionen beträgt derzeit mehr als 771.407.825. Die Zahl der durch COVID-19 verursachten Todesfälle beläuft sich auf mehr als 6,9 Millionen (WHO, Corona Disease (COVID-19) Pandemic: Numbers at glance, 2023).

Die COVID-19-Pandemie hatte im ersten Jahr erhebliche Auswirkungen auf die Bevölkerung in Deutschland. Dies betraf insbesondere Einrichtungen der kritischen Infrastruktur und Krankenhäuser der Maximalversorgung (Bohltken et al., 2020). Viele Beschäftigte des Universitätsklinikums Bonn (UKB) waren aufgrund ihrer Tätigkeit dem höchsten berufsspezifischen Infektionsrisiko ausgesetzt (Bauer et al., 2021).

1.1.1 Epidemische Lage von nationaler Tragweite in Deutschland

Die SARS-CoV-2-Pandemie hat die politischen Instanzen in Deutschland vor die Herausforderung gestellt, den Infektionsschutz, den Arbeitsschutz und die wirtschaftlichen Aktivitäten derart zu regeln, dass ein tragfähiger Kompromiss zwischen den einzelnen Zielsetzungen dieser Gesetze und der Gewährleistung von Sicherheit und Gesundheit bei der Fortführung der Arbeit erreicht werden konnte.

Das Infektionsschutzgesetz (IfSG), konkretisiert durch das „Gesetz zum Schutz der Bevölkerung bei einer epidemischen Lage von nationaler Tragweite“ und die Verordnung zum Schutz vor Neuinfizierungen mit dem Coronavirus SARS-CoV-2 (Coronaschutzverordnung - CoronaSchVO) des Landes Nordrhein-Westfalen vom 22. März 2020, verpflichteten im ersten Jahr der COVID-19-Pandemie alle medizinischen Einrichtungen, die notwendigen Maßnahmen zur Unterbrechung der Infektionsketten und damit zum Schutz der Allgemeinbevölkerung zu implementieren.

Das Arbeitsschutz- und Arbeitssicherheitsgesetz (ArbSchG und ASIG) setzen in Deutschland die EU-Rahmenrichtlinien 89/391/EWG des Rates vom 12. Juni 1989 und 1/383/EWG des Rates vom 25. Juni 1991 um. Diese rechtlichen Regularien zielen darauf ab, die Sicherheit und Gesundheit der Arbeitnehmer bei der Ausübung einer Tätigkeit durch Maßnahmen des Arbeitsschutzes zu gewährleisten und zu verbessern. Arbeitgeber haben nach Maßgabe des Arbeitssicherheitsgesetzes Betriebsärzte und Fachkräfte für Arbeitssicherheit zu bestellen. Diese Experten haben die Aufgabe, den Arbeitgeber beim Arbeitsschutz und bei der Unfallverhütung in allen Fragen des Gesundheitsschutzes zu unterstützen.

Durch das Arbeitsschutzgesetz, das Arbeitssicherheitsgesetz sowie die Biostoffverordnung (BioStoffV) und die Verordnung zur arbeitsmedizinischen Vorsorge (ArbMedVV) als allgemeinverbindliche Regelwerke wird der Schutz der Beschäftigten konkretisiert und in den Mittelpunkt gestellt. Rechtsgrundlage für den Schutz der Bevölkerung vor übertragbaren Krankheiten ist dagegen das Infektionsschutzgesetz (IfSG).

Eine Arbeitsgruppe zur Umsetzung des Pandemieplans wurde während der COVID 19-Pandemie Anfang März 2020 am UKB einberufen. Dieses Gremium konnte die Entwicklungen während der Pandemie zeitnah verfolgen und die notwendigen Maßnahmen zur Bekämpfung des Virus jederzeit festlegen bzw. kurzfristig anpassen. Auf diese Weise wurde der Schutz der Beschäftigten vor infektionsbedingten Berufskrankheiten (BK 3103) sowie arbeitsbedingten Erkrankungen und Gesundheitsgefahren gewährleistet und der Versorgungsauftrag des UKB sichergestellt.

Um Infektionsketten zu unterbrechen, wurden zunächst strenge Hygiene- und Sicherheitsprotokolle sowie Regelungen mit Vermutungswirkung implementiert. In der Folge wurde eine „Corona-Abstrich-Ambulanz“ eingerichtet. In dieser Ambulanz wurde ab März 2020 eine niedrigschwellige Testmöglichkeit eingeführt, bei der unabhängig von klinischen Symptomen, Beschäftigten ein SARS-CoV-2-PCR-Testangebot unterbreitet wurde (Menting et al., 2021). Diese Maßnahme hatte unter anderem das Ziel, die Inzidenz der SARS-CoV-2-Infektionen in den unterschiedlichen Abteilungen, Instituten und Kliniken so schnell wie möglich zu erfassen.

1.1.2 Humane Coronaviren (HCoV) und Impfungen bei beruflicher Indikation

Coronaviren sind humanpathogen und werden nach den Technischen Regeln für biologische Arbeitsstoffe (TRBA) 462 „Einstufung von Viren nach Risikogruppen“ in die Risikogruppen 1 bis 3 eingestuft. Coronaviren sind einzelsträngige umhüllte RNA-Viren mit einem Positivstrang-RNA-Genom. Die Spezies Schweres Akutes Respiratorisches Syndrom Coronavirus Typ 2 (SARS-CoV-2) gehört zur Gattung der Beta-Coronaviren und wird in die Risikogruppe 3 eingestuft (Hasöküsuk et al., 2020, TRBA 462). Eine essenzielle Arbeitsschutzmaßnahme im Rahmen der Primärprävention für Beschäftigte in medizinischen Einrichtungen der Maximalversorgung bei Tätigkeiten mit Infektionsgefährdung im Umgang mit SARS-CoV-2 sind die beruflich indizierten Schutzimpfungen. Impfungen sind Bestandteil der arbeitsmedizinischen Vorsorge und werden den Beschäftigten angeboten, sofern das Risiko einer Infektion tätigkeitsbedingt und im Vergleich zur Allgemeinbevölkerung erhöht ist und der oder die betroffene Beschäftigte nicht bereits über einen ausreichenden Immunschutz verfügt (§ 6 Absatz 2 Satz 3 und 4 ArbMedVV und AMR 6.5).

1.1.3 COVID-19-Impfkampagne am UKB

Ab Januar 2021 konnte der Betriebsärztliche Dienst (BÄD) des UKB eine eigene COVID-19-Impfstelle einrichten. Vorrangiges Ziel war es, den Beschäftigten eine COVID-19-Impfung bei beruflicher Indikation gemäß den Empfehlungen der Ständigen Impfkommission des Robert Koch-Instituts (STIKO-konform) anzubieten.

1.1.4 COVID-19-Impfstoffe

Die beiden erstzugelassenen Impfstoffe basieren auf einer neuartigen mRNA-Technologie, die bisher in keinem der zugelassenen Impfstoffe verwendet wurde (Pardi et al., 2018). Aufbauend auf jahrelanger Grundlagenforschung konnten die beiden Impfstoffe innerhalb eines Jahres nach Ausbruch der COVID-19-Pandemie entwickelt werden (Baden et al. 2020, Pollack et al., 2020) und wurden im Rahmen einer Notfallzulassung in den USA und Europa auf den Markt gebracht (BioNTech, 2021, Europäische Kommission, 2020). Sie sind zur aktiven Immunisierung und zum Schutz vor einem schweren COVID-19-Krankheitsverlauf angezeigt. Die mRNA-Impfstoffe mRNA-1273-Spikevax (Moderna®) und Comirnaty® (BioNTech/Pfizer) werden hinsichtlich ihrer Sicherheit und Wirksamkeit als gleichwertig beurteilt (EMA, 2021). Mittlerweile sind mehr als 300 COVID-19-Impfstoffe in verschiedenen Entwicklungsstadien, von denen die meisten jedoch auf Biotechnologien von Boten-Ribonukleinsäure (mRNA), viralen Vektoren und Proteinuntereinheiten basieren (Barouch, 2022). Die Dynamik der Immunantwort nach einer COVID-19-Impfung sowie nach einer SARS-CoV-2-Infektion mit einer besorgniserregenden Variante (VOC) fällt bei unterschiedlichen Personen, je nach Konstellation, sehr heterogen aus (Dewald et al., 2023, Pušnik et al., 2023, Sette et al., 2021, Hoehl et al., 2023, Gruell et al., 2022, Kaku et al., 2022).

1.1.5 Die adaptive Immunantwort

Im Rahmen der Primärprävention durch Schutzimpfungen spielen B-Zellen (Antikörperbildung), $CD4^+$-T-Zellen (zellvermittelte Immunantwort) und $CD8^+$-T-Zellen (zytotoxische Immunantwort) als Grundkomponenten des adaptiven Immunsystems eine entscheidende Rolle bei der Bekämpfung von Viruserkrankungen im menschlichen Organismus (Sette et al. 2021). Der Impfstoff-induzierte Immunschutz gegen COVID-19 beruht unter anderem auf der Bildung von Antikörpern, welche gegen die Rezeptorbindedomäne des Spike-Proteins gerichtet sind und damit das Andocken des Virus an den zellulären ACE-2-Rezeptor der Wirtszelle verhindern (Baden et al., 2020, Pollack et al., 2020).

1.1.6 Besorgniserregende SARS-CoV-2-Varianten

Plötzliche relevante Veränderungen der molekularen Struktur in der Rezeptorbindungsdomäne des SARS-CoV-2-Virus bergen das Risiko, dass bereits gebildete Antikörper graduell bzw. vollständig ineffektiv werden. Ein weiteres Risiko besteht darin, dass sich mutationsbedingt die Affinität des Virus zum ACE-2-Rezeptor verändert und dieses zu einer geringeren Neutralisationsaktivität gegen SARS-CoV-2 führt (Guruprasad, 2021, Davis-Gardner et al., 2023).

Seit November 2020 wird weltweit die Entstehung einer zunehmenden Zahl an Virusvarianten mit derartigen Mutationen (VOC) registriert (**Abb. 1**) und weiterverfolgt (WHO 2023, Robert Koch-Institut, „Aktualisierter Bericht zu Virusvarianten von SARS-CoV-2 in Deutschland, insbesondere zur Variant of Concern (V.o.C.) B.1.1.7“, 2022).

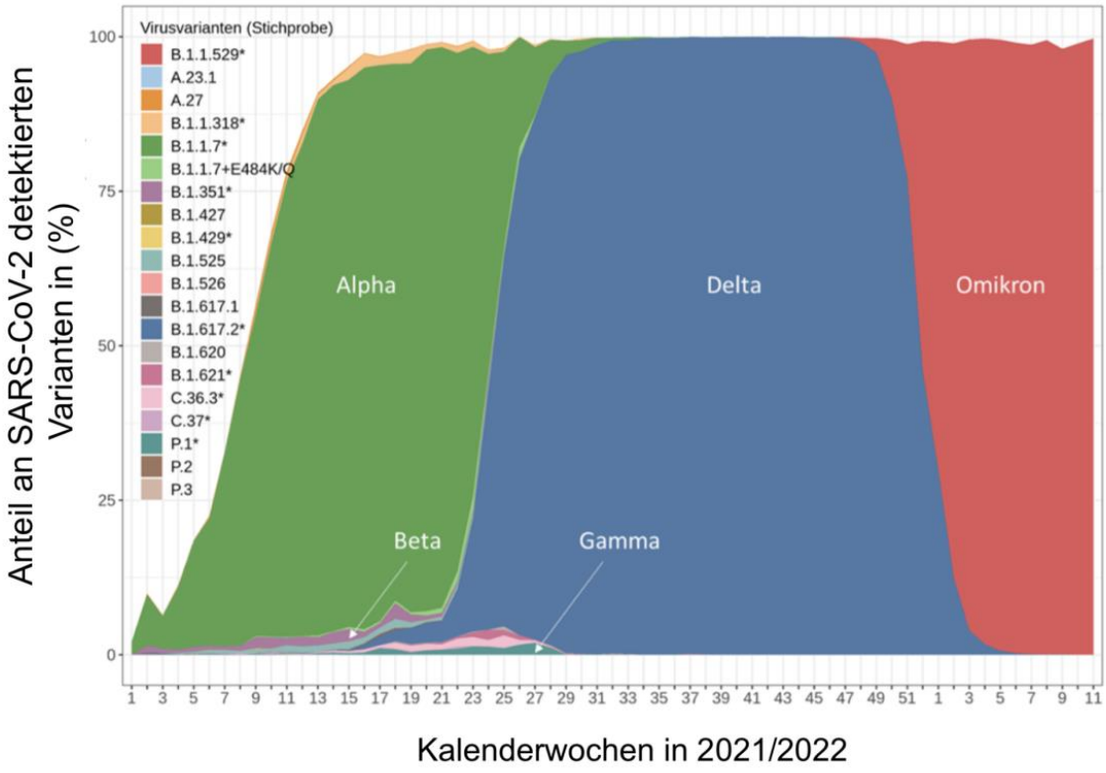

Abb. 1: SARS-CoV-2-Varianten-Verteilung in Deutschland in den Kalenderwochen 1/2021 bis 11/2022 (modifiziert nach Robert Koch-Institut, 2022)

Anteil der detektierten Virusvarianten mit derartigen Mutationen, sog. VOC - B.1.1.7, B.1.617.2 und B1.1.529 (Alpha-, Delta-, Omikronvariante) registriert und weiterverfolgt im ersten und zweiten Jahr der COVID-19-Pandemie (WHO 2023, Robert Koch-Institut, 2023).

Aus diesem Grund ist eine prospektive und differenzierte Untersuchung der Dynamik der adaptiven Immunantwort gegen SARS-CoV-2 notwendig, um die Wirksamkeit von Impfstoffen gegen COVID-19 besser zu verstehen.

1.1.7 Fragestellung

Zu Beginn der COVID-19-Pandemie lagen keine Daten über die Wirksamkeit des mRNA-Impfstoffs mRNA-1273-Spikevax von Moderna® im Hinblick auf die neuen besorgniserregenden SARS-CoV-2-Varianten im Rahmen von Langzeitstudien vor (Baden et al., 2021). Das Risiko einer Umgehung der erlangten Immunität (immune escape) durch VOC wurde in der Diskussion unterschiedlich bewertet (Wibmer et al., 2021).

Am UKB wurden die ersten 4.100 Beschäftigten mit dem neuen Impfstoff mRNA-1273-Spikevax von Moderna® grundimmunisiert. Vor diesem Hintergrund beschloss der Betriebsärztliche Dienst (BÄD) interdisziplinär mit dem Institut für Virologie des UKB im Rahmen einer Promotionsarbeit eine prospektive infektionsepidemiologische “Real World Data” (RWD)-basierte Studie durchzuführen.

Die hier vorliegende „Moderna-Studie“ bei Beschäftigten mit erhöhter berufsspezifischer Exposition am UKB untersuchte die Wirksamkeit der mRNA-COVID-19-Impfstoffe (Comirnaty® von BioNTech/Pfizer und 1273-Spikevax von Moderna®*)* in Bezug auf die kumulative Inzidenz (KI) von SARS-CoV-2-Infektionen mit den VOC: B.1.1.7, B1.1.529 und B.1.617.2 im Zeitraum vom 01.03.2021 bis zum 28.02.2022 (**Abb. 2**).

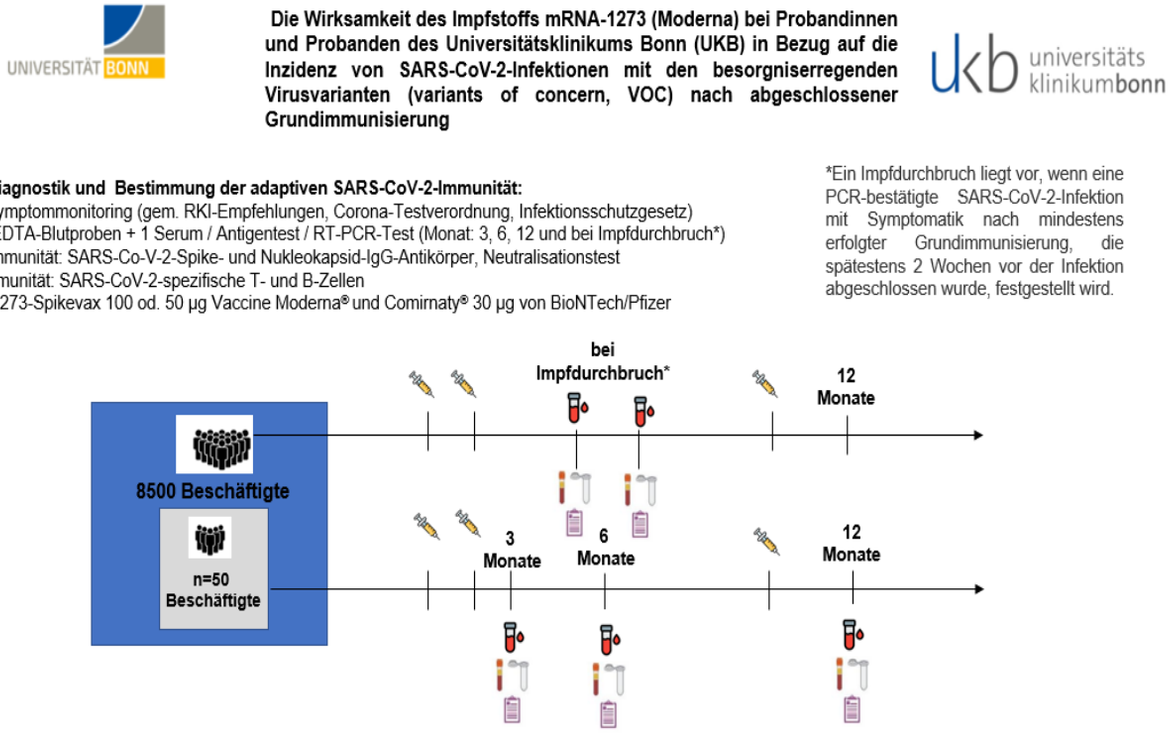

Abb. 2: Studiendesign – Die „Moderna-Studie“ am UKB

Um die Langlebigkeit und Wirksamkeit der adaptiven Immunantwort nach Grundimmunisierung mit mRNA-1273-Spikevax (Moderna®) und der ersten Auffrischimpfung mit einem mRNA-COVID-19-Impfstoff differenziert zu untersuchen, wurden im ersten Arm der Studie die zellulären und humoralen Parameter der adaptiven Immunantwort bei 50 Probandinnen und Probanden über einen Zeitraum von zwölf Monaten erfasst (modifiziert nach Monzón-Posadas et al., 2023). Darüber hinaus wurden Beschäftigte des UKB, die an einer Durchbruchsinfektion erkrankten, über die “Corona-Abstrichambulanz“ in den zweiten Arm der Studie eingeschlossen. Eine weitere Gruppe von 27 Beschäftigten wurde rekrutiert, um die Dynamik der Immunantwort unmittelbar nach der Auffrischimpfung zu verfolgen. Endpunkte der Studie sind die kumulative Inzidenz von SARS-CoV-2-Infektionen sowie die Erfassung der Heterogenität der adaptiven Immunantwort.

1.2 Material und Methoden

Insgesamt wurden 77 Probandinnen und Probanden rekrutiert. Sie stammen aus einem Kollektiv von 4.100 Mitarbeitenden, die im Zuge der Priorisierung der Impfkampagne (analog zur Priorisierung nach der Corona-Impfverordnung) der Kategorie A zugeordnet waren und ein Impfangebot am UKB erhielten (von insgesamt ca. 8500 Beschäftigten des UKB zwischen dem 01.03.2021 und dem 14.04.2021).

1.2.1 Studienkohorte: mRNA-Impfstoff-Studie am UKB 2021-2023

Die Beschäftigten waren einem besonders hohen berufsspezifischen Infektionsrisiko mit SARS-CoV-2 ausgesetzt. Die Anzahl der Probandinnen und Probanden wurde nach medizinisch-statistischer Beratung durch das Institut für Medizinische Biometrie des UKB festgelegt, um statistisch signifikante Ergebnisse zu erzielen. Zu den Tätigkeitsbereichen der Kategorie A zählten u. a. folgende Kliniken, Abteilungen und Zentren: das interdisziplinäre Notfallzentrum (INZ), alle Intensivstationen, die COVID-19-Stationen, die Dialysestation, alle operativen Fächer, die Kliniken für Pneumologie, Gastroenterologie, Anästhesiologie, Zahnheilkunde, Hals-Nasen-Ohren-Heilkunde, Radiologie, das Zentrum für Physiotherapie, die Transport-, Reinigungs- und Servicedienste, die Studierenden der Zahn- und Humanmedizin im Praktischen Jahr sowie Mitarbeitende mit engem Kontakt zu vulnerablen Gruppen (Transplantationsmedizin, Hämato-Onkologie).

Mit Hilfe eines Zufallsgenerators wurden zunächst 50 Probandinnen und Probanden rekrutiert, die initial zwei Dosen des mRNA-basierten COVID-19-Impfstoffs 1273-Spikevax von Moderna® (100 µg) zur Grundimmunisierung erhielten. Sechs Monate später wurde den Probandinnen und Probanden eine erste Auffrischimpfung mit einem mRNA-basierten COVID-19-Impfstoff (Comirnaty® 30 µg von BioNTech/Pfizer oder 1273-Spikevax 50 µg Vaccine Moderna®) verabreicht.

Zusätzlich wurde eine weitere Gruppe von 22 Beschäftigten (naiv gegenüber SARS-CoV-2) und 5 Beschäftigten (die eine Durchbruchsinfektion mit SARS-CoV-2 erlitten hatten - Hybridimmunität), rekrutiert, um die Dynamik der Immunantwort unmittelbar nach der Auffrischimpfung zu untersuchen. Bei diesen Personen wurde die Immunantwort drei und 15 Wochen nach der Auffrischimpfung untersucht. Die erste Auffrischimpfung erfolgte zum selben Zeitpunkt mit einem in der EU-zugelassenen mRNA-COVID-19-Impfstoff.

Alle Studienteilnehmenden waren zum Zeitpunkt der Laborprobenentnahme Beschäftigte oder Studierende des Universitätsklinikums Bonn (**Abb. 3**). Die Durchbruchsinfektionen wurden mittels RT-PCR über die „Corona-Abstrich-Ambulanz“ im Rahmen des Routine-Screenings durch die Diagnostik-Abteilung des Instituts für Virologie nachgewiesen und serologisch mittels Anti-Nukleokapsid-ELISA bestätigt. Die Probandinnen und Probanden mit Durchbruchsinfektionen konnten über den zweiten Arm der Studie rekrutiert werden, um die kumulative Inzidenz von SARS-CoV-2-Infektionen und die Belastbarkeit der Hybridimmunität nach Infektion mit den dominanten VOC zu quantifizieren (Pušnik et al. 2023).

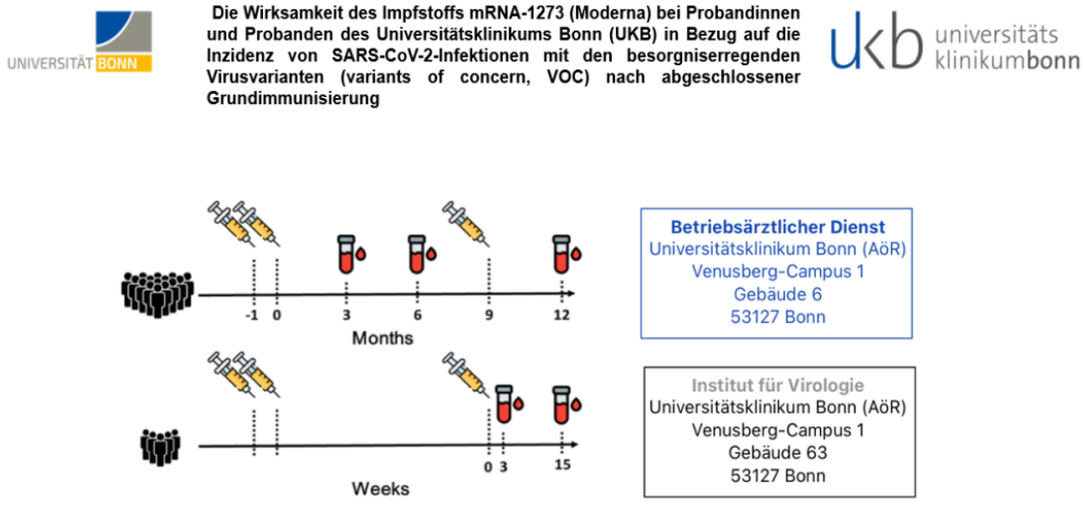

Abb. 3: Studienprotokoll „Moderna-Studie“ am UKB
Es wurden 50 Probandinnen und Probanden rekrutiert, die zunächst mit zwei Dosen eines mRNA-basierten COVID-19-Impfstoffs (1273-Spikevax 100 µg von Moderna®) geimpft wurden und sechs Monate später eine erste Auffrischimpfung mit einem mRNA-basierten COVID-19-Impfstoff (Comirnaty® 30 µg von BioNTech/Pfizer oder 1273-Spikevax 50 µg (Vaccine Moderna®) erhielten. Bei 27 Beschäftigten erfolgte die Erfassung der Immunantwort drei und 15 Wochen nach der Booster-Impfung (modifiziert nach Monzón-Posadas et al., 2023).

Die Studienteilnehmer stellten periphere Blutproben, Speichelproben und Rachenabstriche zur Verfügung. Die qualitativen Daten wurden mittels eines Fragebogens erfasst (siehe Anhang).

Das Blut wurde zentrifugiert und das EDTA-Plasma bis zur Analyse bei - 80°C gelagert. Vor der Speichelentnahme wurden die Probandinnen und Probanden angewiesen,

mindestens 60 Minuten lang nichts zu essen oder zu trinken, den Speichel für 1 - 2 Minuten zurückzuhalten und in ein Zentrifugenröhrchen auszuspucken. Um feste Partikel zu entfernen, wurden die Speichelproben zentrifugiert und anschließend bei - 20 °C eingefroren. Die mononukleären Zellen des peripheren Blutes (PBMC) wurden durch Dichtegradientenzentrifugation isoliert und in flüssigem Stickstoff kryokonserviert (Monzón-Posadas et al., 2023).

1.2.2 Votum der Ethikkommission

Die Stellungnahme der Ethikkommission der Medizinischen Fakultät der Universität Bonn vor Beginn der Studie ergab keine berufsethischen oder juristischen Bedenken gegen die Durchführung der Studie (siehe Anlage: Aktenzeichen 125/21). Alle Probandinnen und Probanden wurden aufgeklärt und gaben eine schriftliche Einverständniserklärung ab, die von der Ethikkommission der Medizinischen Fakultät der Universität Bonn positiv begutachtet wurde. Die Untersuchungen wurden auf der Basis der revidierten Deklaration von Helsinki des Weltärztebundes (1983) und nach den entsprechenden gesetzlichen Vorgaben durchgeführt.

1.2.3 Bestimmung von SARS-CoV-2-S1-spezifischem IgG im Blutplasma

Die Titer der SARS-CoV-2-S1-spezifischen IgG-Antikörper wurden mit Hilfe eines eigens dafür entwickelten quantitativen ELISA bestimmt. Zur Bestimmung des Referenzbereichs wurden 30 Plasmaproben von Personen untersucht, die zu keinem Zeitpunkt mit SARS-CoV-2 infiziert waren. Auf der Grundlage der Messungen wurde der Cutoff-Wert (Mittelwert + 2 x Varianz) bestimmt, ab dem die Ergebnisse als positiv für SARS-CoV-2-S1-IgG-AK angesehen werden: 19,2 Binding Antiköper Units BAU/ml (Monzón-Posadas et al., 2023).

1.2.4 Bestimmung von SARS-CoV-2-S1-Antikörper im Speichel

Die SARS-CoV-2-S1-spezifischen IgA- und IgG-Antikörpertiter im Speichel der Testpersonen wurden mit einem in-House quantitativen ELISA gemessen (Monzón-Posadas et al., 2023). Diese Einheiten sind nicht mit denen des Plasma-ELISA vergleichbar. Zur Bestimmung der Referenzbereiche wurden Speichelproben von 24 Personen gemessen, die seronegativ für anti-SARS-CoV-2 Spike IgG waren. Anhand der

Messungen wurde der Cutoff-Wert ermittelt, ab dem die Ergebnisse als positiv angesehen wurden: 0,014 WE für IgG und 0,012 WE für IgA (Monzón-Posadas et al., 2023).

1.2.5 Plaque-Reduktions-Neutralisationstest

Die Neutralisationskapazität des Blutplasmas wurde mit Hilfe eines Plaque-Reduktions-Neutralisationstests ermittelt. Mit Hilfe der GraphPad Prism Software wurde die Anzahl der Plaques gegen die Serumverdünnungen aufgetragen und die mittlere Hemmkonzentration (IC50) gemessen (Monzón-Posadas et al., 2023).

1.2.6 Isolierung von B-Zellen

Der Nachweis von SARS-CoV-2-S1-spezifischen B-Gedächtnis-Zellen erfolgte mittels Durchflusszytometrie (BD FACS Celesta^{TM}) durchgeführt von Herrn Dr. J. Pušnik, siehe Monzón-Posadas et al., 2023, Pušnik et al., 2023.

1.2.7 Ex-vivo-Stimulation von T-Zellen

Die Untersuchung von SARS-CoV-2-S1-spezifischen T-Gedächtnis-Zellen erfolgte mittels Durchflusszytometrie (BD FACS Celesta^{TM}) durchgeführt von Herrn Dr. J. Pušnik, siehe Monzón-Posadas et al., 2023, Pušnik et al., 2023.

1.2.8 Nachweis von SARS-CoV-2-spezifischen T-Zellen

Der Nachweis von SARS-CoV-2-S1-spezifischen T-Gedächtnis-Zellen erfolgte mittels Durchflusszytometrie (BD FACS Celesta^{TM}) durchgeführt von Herrn Dr. J. Pušnik, siehe Monzón-Posadas et al., 2023, Pušnik et al., 2023.

1.2.9 Statistische Auswertung

Folgende Anmerkungen kennzeichnen die statistische Signifikanz: *p<0,05, **p<0,01, ***p<0,001, ****p<0,0001 (Monzón-Posadas et al., 2023). Die gesammelten Daten wurden mit dem Statistikprogramm R statistisch ausgewertet. Die Korrelationsstärken wurden mit dem Spearman-Test bestimmt. Die Unterschiede zwischen den Gruppen wurden mit dem Wilcoxon-Test für gepaarte Daten mit der Korrektur für multiples Testen nach Holm (Weiß, 2019, S. 134) bewertet.

1.3 Ergebnisse

Im Rahmen der „Moderna-Studie“ am UKB wurde die Wirksamkeit der adaptiven humoralen und zellulären Immunität sowie die kumulative Inzidenz von SARS-CoV-2-Infektionen mit den besorgniserregenden Varianten nach Grundimmunisierung und erster Booster-Impfung im Langzeitverlauf untersucht (**Tab.1-4, Abb. 4-6**).

1.3.1 SARS-CoV-2-Fälle am UKB vom 01.03.2021 bis zum 28.02.2022

Tab. 1: Anzahl der SARS-CoV-2-RT-PCR-positiven Fälle „Corona-Abstrich-Ambulanz“ des UKB

Monat, Jahr	SARS-CoV-2 positiv getestete Fälle mittels RT-PCR: UKB-Beschäftigte	SARS-CoV-2 positiv getestete Fälle mittels RT-PCR: „Moderna-Studie“
März, 2021	24	0
April, 2021	16	0
Mai, 2021	4	0
Juni, 2021	2	0
Juli, 2021	5	0
August, 2021	9	0
September, 2021	24	0
Oktober, 2021	24	0
November, 2021	73	0
Dezember, 2021	87	0
Januar, 2022	353	3
Februar, 2022	448	4
Gesamt:	1069	7

Die Anzahl der SARS-CoV-2-positiven Fälle bei Beschäftigten des UKB (Gesamtzahl = UKB-Beschäftige + externe getestete Personen) wurde, während der COVID-19-Pandemie in der „Corona-Abstrich-Ambulanz“ des Betriebsärztlichen Dienstes des UKB zwischen dem 16.03.2020 und 06.01.2023 erfasst (**Abb. 4 und 5**).

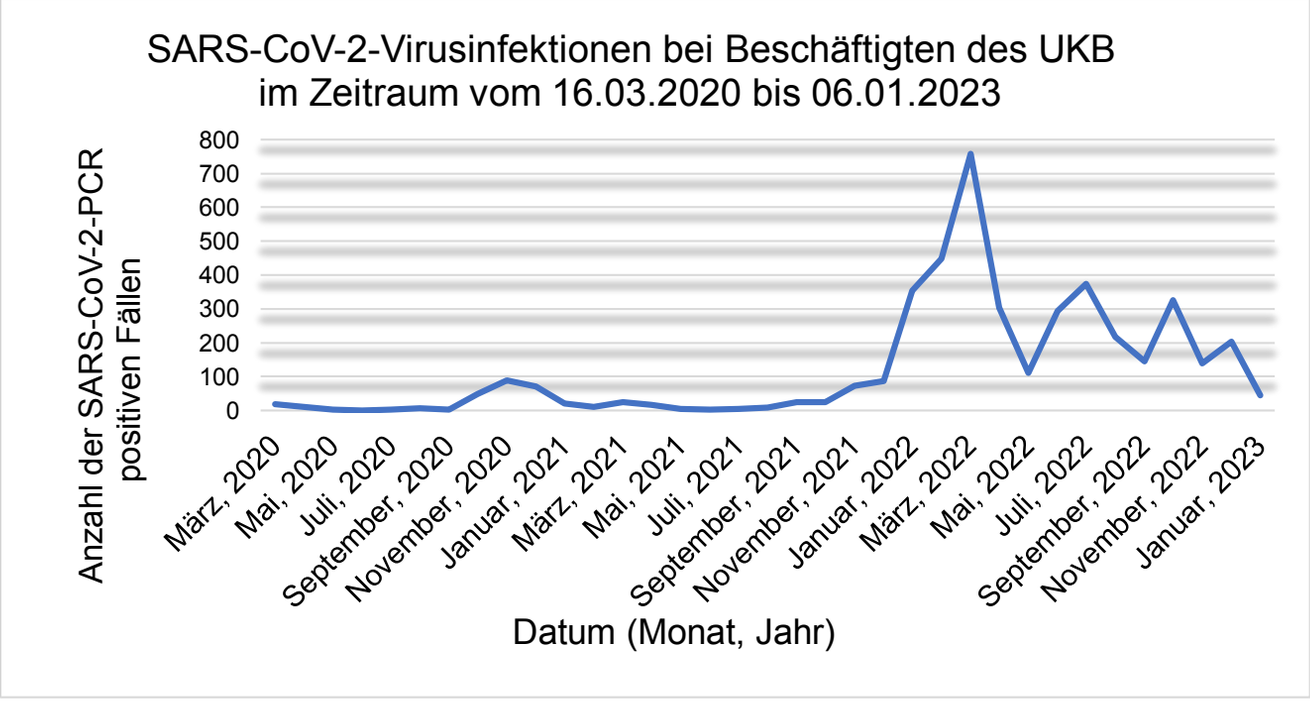

Abb. 4: Die Infektionswelle am UKB im Verlauf der COVID-19-Pandemie

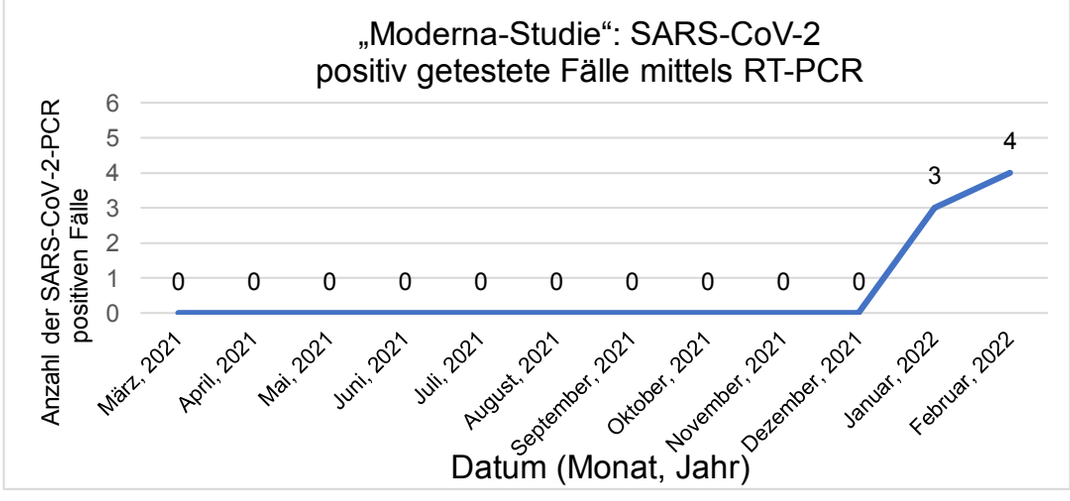

Abb. 5: SARS-CoV-2-Infektionsfälle bei Probandinnen und Probanden der „Moderna-Studie“ am UKB in der Zeitspanne vom 01.03.2021 bis zum 28.02.2022

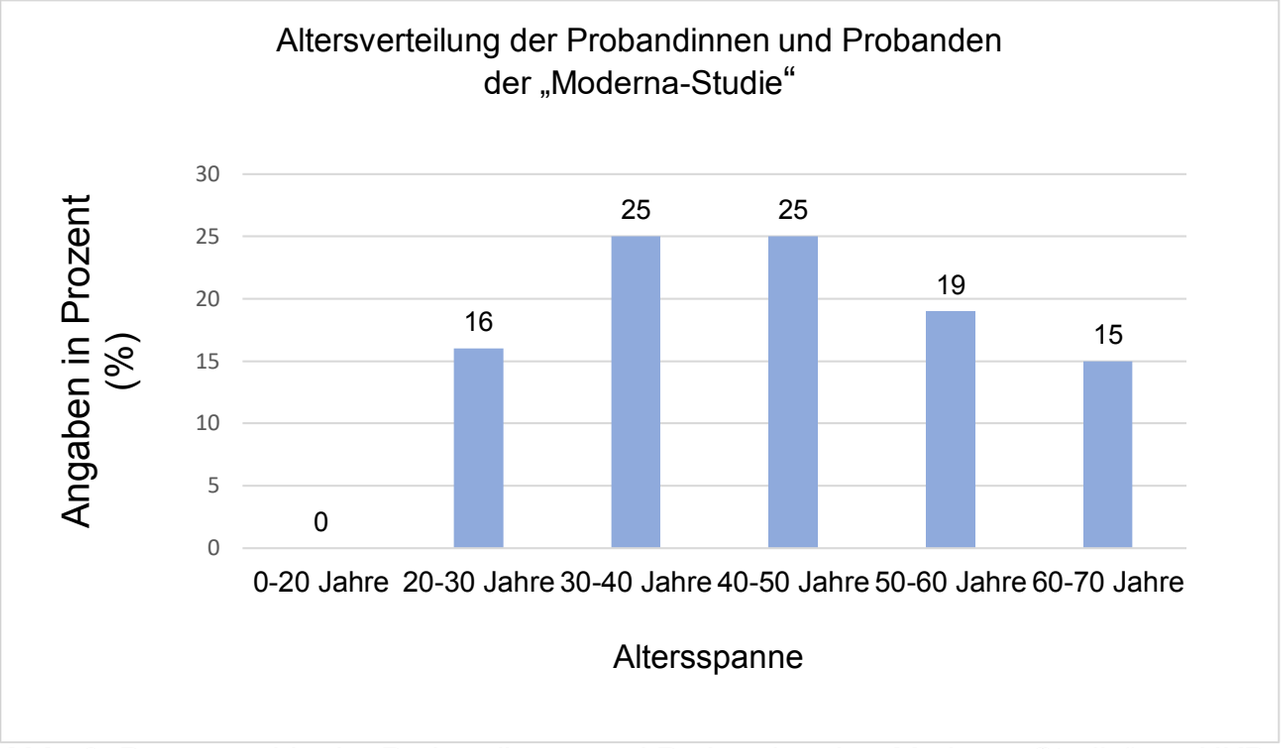

Abb. 6: Demographie der Probandinnen und Probanden der „Moderna-Studie“ am UKB - nach Altersgruppen in Prozentangaben

1.3.2 Kumulative Inzidenz vom 01.03.2021 bis zum 28.02.2022

Tab. 2: Kumulative Inzidenz von SARS-CoV-2-Infektionen im Zeitraum vom 01.03.2021 bis 28.02.2022

Kumulative Inzidenz SARS-CoV-2-Infektionen Beobachtungszeit: 01.03.2021-28.02.2022	Prozent (%)
Kumulative Inzidenz bei Probandinnen und Probanden der „Moderna-Studie“	14

Tab. 3: Demographische Darstellung der Kohorte der SARS-CoV-2-infizierten Probandinnen und Probanden der “Moderna-Studie” am UKB zwischen 01.03.2021 und 28.02.2022 nach Geschlecht in Prozentangaben

Geschlecht	Prozent (%)
weiblich	54
männlich	46
divers oder ohne Angabe	0

Die Durchbruchsinfektionen wurden mittels RT-PCR über die „Corona-Abstrich-Ambulanz“ im Rahmen des Routine-Screenings durch die Diagnostik-Abteilung des Instituts für Virologie nachgewiesen (**Tab. 4**).

Tab. 4: SARS-CoV-2 Infektionen bei Probandinnen und Probanden der „Moderna-Studie“ am UKB zwischen dem 01.03.2021 und dem 28.02.2022 nach VOC in Prozentangaben

SARS-CoV-2-Variante (VOC)	Angaben in Prozent (%)
B.1.1.7 (Alpha)	33
B.1.617.2 (Delta)	17
B.1.1.529 (Omikron)	50

1.3.3 SARS-CoV-2-S1-spezifische Antikörperreaktion im Blutplasma

Zur Erfassung der Dynamik der SARS-CoV-2-S1-spezifischen Antikörperantwort im Blutplasma wurden im Verlauf der „Moderna-Studie“ am UKB die Plasmaspiegel der SARS-CoV-2-IgG-Antikörper (S1-Untereinheit-spezifisch) bei den Probandinnen und Probanden quantifiziert (**Abb. 7**).

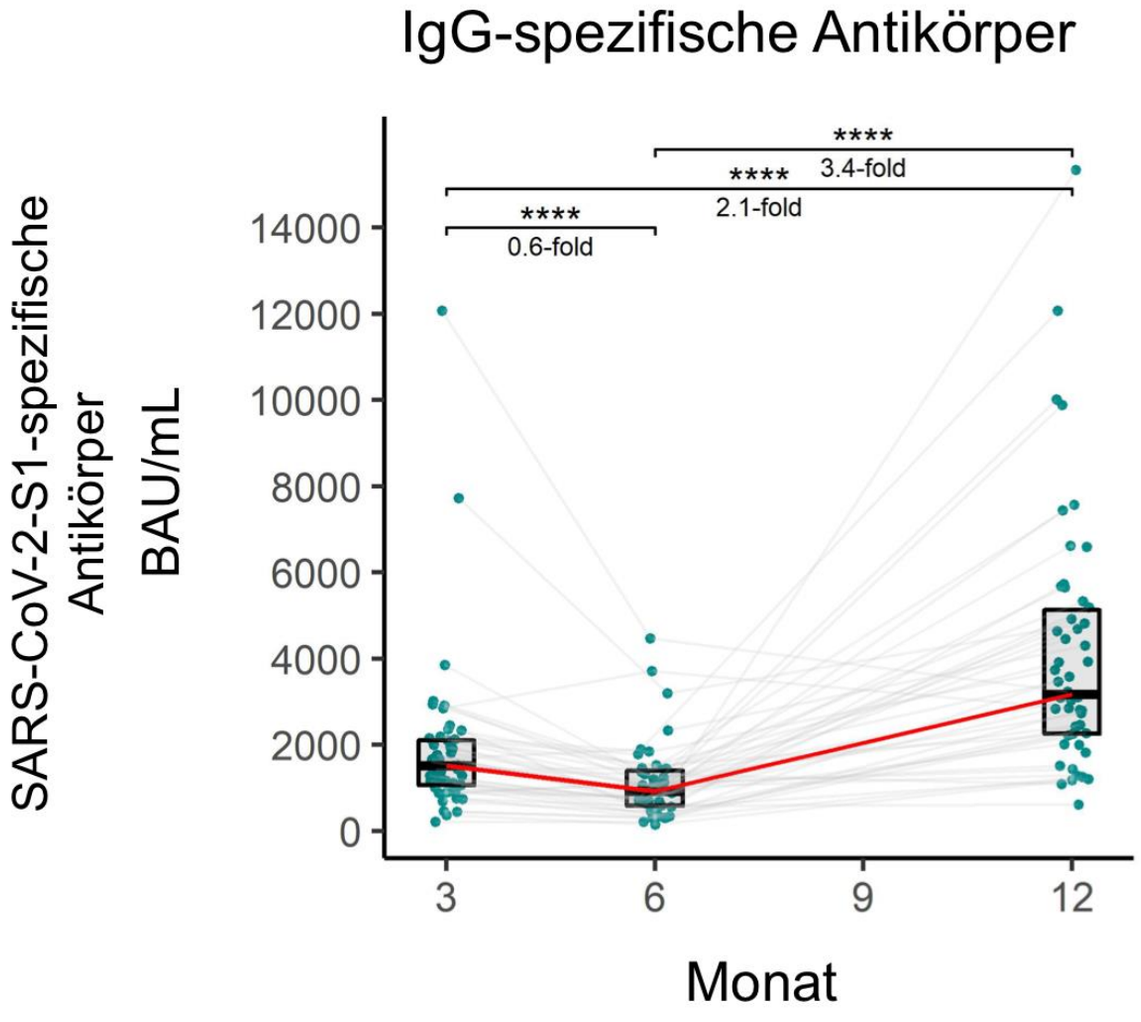

Abb. 7: Impfinduzierte Antikörper (Anti-Spike-IgG-AK) bei Probandinnen und Probanden im Verlauf (modifiziert nach Monzón-Posadas et al., 2023)

Die Ergebnisse zeigen, dass der Gehalt an S1-spezifischem IgG-Antikörper zwischen dem dritten und sechsten Monat nach der Impfung mit zwei Dosen des mRNA-Impfstoffs um das 1,7-fache abnimmt ($p<0,0001$) und nach der Auffrischimpfung zwischen dem sechsten und zwölften Monat ansteigt.

Neutralisierende Antikörper sind das am besten definierte Korrelat des Schutzes vor einer SARS-CoV-2-Infektion (Feng et al., 2021). Um die Dynamik der neutralisierenden Antikörper zu beurteilen, wurden Plaquereduktions-Neutralisationstests mit lebenden, nicht manipulierten SARS-CoV-2-Isolaten (Wildtyp-, Delta- und Omikron-Variante) durchgeführt (**Abb. 8**).

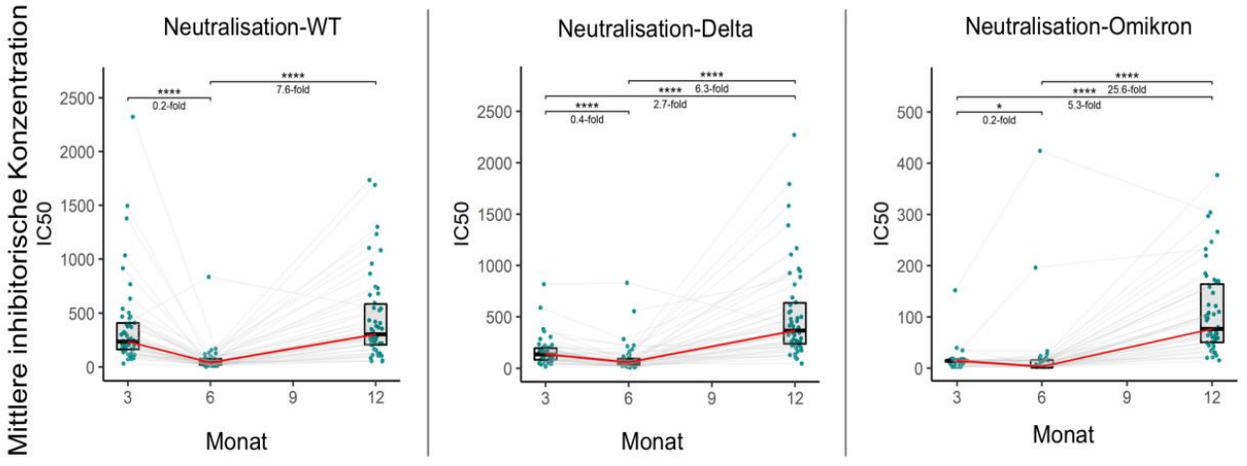

Abb. 8: Dynamik der neutralisierenden Antikörper (modifiziert nach Monzón-Posadas et al., 2023)

Die Neutralisierungskapazität gegen das Wildtyp-Virus nahm in den Monaten drei und sechs um das Fünffache ab (P<0,0001). Bei den Delta- und Omikron-Varianten betrug der Rückgang der Neutralisationskraft das 2,5- bzw. 5-Fache (P<0,0001, P<0,05). Im zwölften Monat, also etwa drei Monate nach der Auffrischimpfung, stiegen die Neutralisierungswerte für die Wildtyp-, Delta- und Omikronvariante im Vergleich zum sechsten Monat um das 7,6-, 6,3- bzw. 25,6-fache (P<0,0001, P<0,0001, P<0,0001) (Monzón-Posadas et al., 2023).

Zwischen der 3. und 15. Woche nach der Auffrischungsimpfung nahm der Gehalt an S1-spezifischen IgG-AK gegen die Wildtyp-Variante um den Faktor 3,3 ab (P < 0,0001). Die Neutralisationskapazität verringerte sich gegenüber der Variante Delta um den Faktor 2 (P < 0,01) und gegenüber der Variante Omikron um den Faktor 2,5 (P < 0,001) (**Abb. 9**).

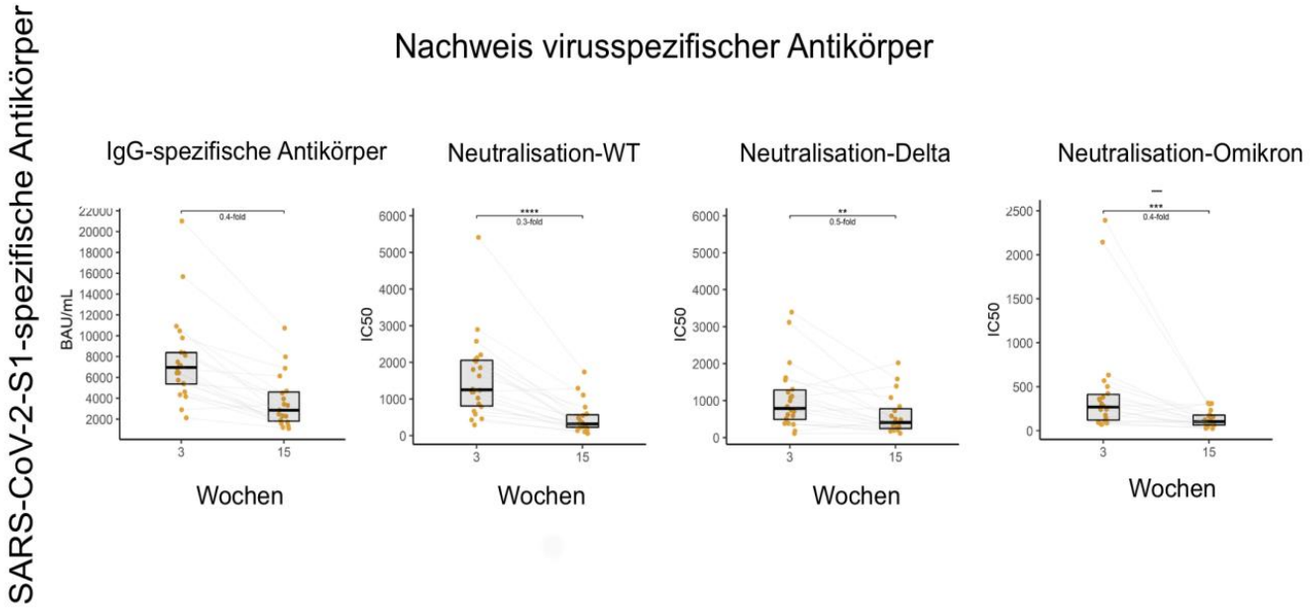

Abb. 9: Abnahme der Neutralisationskapazität zwischen den Wochen drei und 15 nach der Auffrischimpfung (modifiziert nach Monzón-Posadas et al., 2023)

Um die unterschiedliche Neutralisationssensitivität der SARS-CoV-2-Varianten durch Seren von Probandinnen und Probanden der Studie zu demonstrieren, wurde anschließend die Plasmaneutralisationskapazität der Wildtyp-, Delta- und Omikronvariante in den Monaten drei, sechs und zwölf nach vollständiger Grundimmunisierung und Auffrischimpfung direkt verglichen. Zum ersten Zeitpunkt war die Wildtyp-Variante am anfälligsten für die Neutralisation, gefolgt von der Delta-Variante, während sich die Omikron-Variante als deutlich resistenter erwies. Allerdings neutralisierten die Antikörper im Blutplasma der geimpften Personen nach 6 Monaten die Delta-Variante effizienter als die Wildtyp-Variante. Die Omikron-Variante blieb zu allen Zeitpunkten am widerstandsfähigsten gegen die Neutralisierung, seine Anfälligkeit für die Neutralisierung nahm jedoch nach der Auffrischimpfung signifikant zu (**Abb. 10**) (Monzón-Posadas et al., 2023).

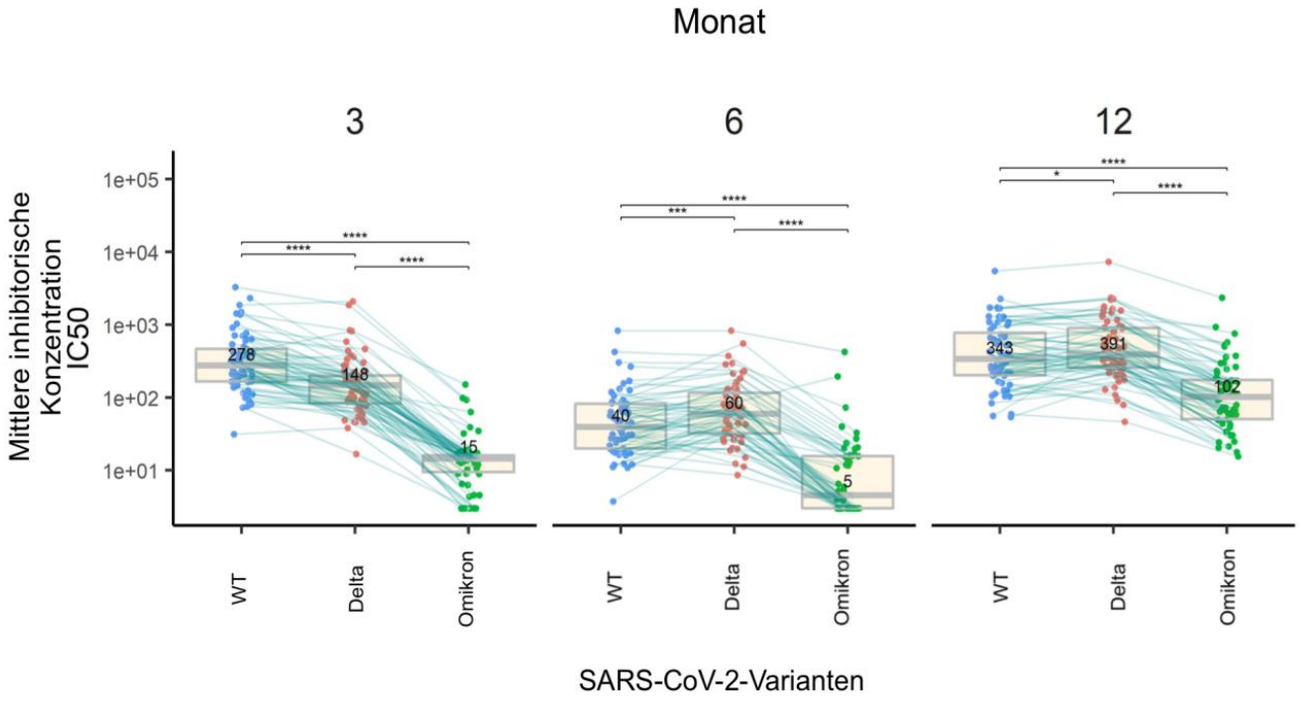

Abb. 10: Anfälligkeit der SARS-CoV-2-Varianten für die Neutralisierung (modifiziert nach Monzón-Posadas et al., 2023)

Insgesamt zeigen die Ergebnisse, dass die anfängliche Antikörperreaktion gegen SARS-CoV-2 nach den beiden ersten Impfdosen mit mRNA-1273-Spikevax von Moderna®, aber auch nach der ersten Auffrischimpfung mit einem mRNA-Impfstoff nachlässt. Unsere Daten zeigen eine erhöhte Wirksamkeit der Neutralisierung nach der ersten Auffrischimpfung gegen die oben genannten SARS-CoV-2-Varianten durch die Seren der Probandinnen und Probanden im Vergleich zu nur zwei Impfstoffdosen (**Abb.10**) (Monzón-Posadas et al., 2023).

1.3.4 SARS-CoV-2-spezifische Antikörperspiegel im Speichel

Die Auffrischimpfung bewirkte einen Anstieg der SARS-CoV-2-spezifischen Antikörperspiegel im Speichel der Probandinnen und Probanden. Als „respiratorisches Virus“ infiziert SARS-CoV-2 zunächst die oberen Atemwege (V'kovski et al., 2021), so dass eine Infektion mit SARS-CoV-2 durch das Vorhandensein von Antikörpern in der Schleimhaut der oberen Atemwege sowie in Schleim und Speichel abgewehrt werden kann.

Aus diesem Grund wurden die Titer von SARS-CoV-2-S1-spezifischem IgG- und IgA-Antikörper mit einem ultrasensitiven ELISA in Speichelproben, die aus dem Rachenraum der Probandinnen und Probanden stammen, gemessen. Die Ergebnisse zeigen, dass die SARS-CoV-2-S1-spezifischen IgG-AK-Spiegel im Speichel zwischen dem dritten und sechsten Monat nach der Grundimmunisierung um das 2,5-fache abnehmen ($P<0,0001$). Nach einer Auffrischimpfung (neun Monate nach der ersten Impfung) stiegen die SARS-CoV-2-S1-spezifischen IgG-AK-Spiegel im Monat zwölf im Vergleich zu Monat sechs um das 8,4-fache an ($P<0,0001$) (Monzón-Posadas et al., 2023).

1.3.5 SARS-CoV-2-spezifische B-Gedächtnis-Zellen

Die Konzentration der SARS-CoV-2-spezifischen B-Gedächtnis-Zellen nimmt sowohl im Laufe der Zeit als auch nach einer Auffrischimpfung (Booster-Impfung) zu (Pušnik et al., 2023). In Studien konnte nachgewiesen werden, dass die Anzahl von SARS-CoV-2-spezifischen B-Gedächtnis-Zellen nach einer Infektion oder Impfung viel länger auf einem erhöhten Niveau bleiben als die Antikörpertiter, weshalb sie besonders relevant für das immunologische Langzeitgedächtnis sind (Pušnik et al., 2022, Terreri et al., 2022).

Die Konzentration von SARS-CoV-2-S1-spezifischen B-Gedächtnis-Zellen im peripheren Blut von geimpften Personen konnte zu jedem Zeitpunkt der Probenahme mit Hilfe der Multiparameter-Durchflusszytometrie gemessen werden. Die Konzentration von IgA^+- und IgM^+-SARS-CoV-2-S1-spezifischen B-Gedächtnis-Zellen blieb zwischen den Monaten drei, sechs und zwölf stabil (Monzón-Posadas et al., 2023). Die meisten Personen hatten nachweisbar mehr IgG^+- als IgM^+-SARS-CoV-2-S1-spezifische B-Gedächtnis-Zellen. IgA^+-SARS-CoV-2-S1-spezifische B-Gedächtnis-Zellen konnten nur bei sehr wenigen Personen nachgewiesen werden.

1.3.6 SARS-CoV-2-spezifische $CD4^+$-T-Zell-Reaktionen

CD4+ T-Zellen spielen durch die Regulation der Antikörperproduktion eine Schlüsselrolle in der impfstoffinduzierten Immunantwort (Painter et al., 2021; Todryk, 2018). Diese Zellen können nicht nur stimulierend und koordinierend wirken, sondern auch Zellgifte freisetzen und infizierte Zellen direkt zerstören (Takeuchi, 2017).

Unsere Analysen haben gezeigt, dass die SARS-CoV-2-spezifischen $CD4^+$-T-Zell-Antworten von langer Dauer sind und durch die Booster-Impfung nur in geringem Maße verstärkt werden (Monzón-Posadas et al., 2023).

1.3.7 SARS-CoV-2-spezifische $CD8^+$-T-Zell-Reaktionen

Die SARS-CoV-2-spezifischen $CD8^+$-T-Zell-Reaktionen sind dauerhaft und werden durch die Auffrischimpfung ebenfalls mäßig verstärkt (Monzón-Posadas et al., 2023; Pušnik et al., 2023). $CD8^+$-T-Zellen sind in der Lage, SARS-CoV-2-virusinfizierte Zellen zu erkennen und abzutöten und stellen somit einen wichtigen antiviralen Schutzmechanismus dar. In Studien konnte nachgewiesen werden, dass SARS-CoV-2-spezifische $CD8^+$-T-Zellen die Infektion erfolgreich begrenzen und mit dem Schutz vor schwerer Krankheit positiv korrelieren (Moss, 2022, Kundu et al., 2022).

Bei einem Vergleich zwischen $CD8^+$- und $CD4^+$-S-spezifischen-T-Zell-Reaktionen zeigte sich, dass $CD4^+$-T-Zellen im Allgemeinen häufiger vorhanden waren als $CD8^+$-T-Zellen. Der höchste Anteil an $CD8^+$-T-Zellen wurde bei den zytotoxischen T-Zellen beobachtet, gefolgt von IFNγ-exprimierenden, TNFα-exprimierenden und IL2-exprimierenden SARS-CoV-2-S-spezifischen T-Zellen. Für keine der Funktionen wurden signifikante Unterschiede zwischen den drei Messzeitpunkten festgestellt. Der Anteil der Probandinnen und Probanden mit nachweisbaren SARS-CoV-2-S-spezifischen T-Lymphozyten war bei allen Funktionen und zu allen drei Zeitpunkten bei den $CD4^+$-T-Lymphozyten höher als bei den $CD8^+$-T-Lymphozyten (Monzón-Posadas et al., 2023; Pušnik et al., 2023).

1.4 Diskussion

Die Ergebnisse der am UKB durchgeführten prospektiven infektionsepidemiologischen "Moderna-Studie" auf der Basis von "Real World“-Daten (RWD) passen zu den Ergebnissen anderer publizierter Studien und Meta-Analysen.

1.4.1 mRNA-Impfstoffe und die adaptive Immunantwort

Derzeit besteht ein wissenschaftlicher Konsens darüber, dass COVID-19 mRNA-basierte Impfstoffe eine robuste adaptive Immunantwort induzieren (Polack et al., 2020, Baden et al., 2020, Turner et al., 2021, Walsh et al., 2020), die jedoch, wie die durch eine SARS-CoV-2-Infektion ausgelöste Immunität, nur von begrenzter Dauer ist (Pérez-Alós et al., 2022, Levin et al., 2021). Neben der Entstehung neuer, besorgniserregender Virusvarianten ist dies einer der Hauptgründe für die Inzidenz von SARS-CoV-2-Infektionen bei UKB-Beschäftigten trotz hoher Seroprävalenz von SARS-CoV-2-Antikörpern während der COVID-19-Pandemie.

1.4.2 Die “Moderna-Studie“ am UKB

Unsere Daten zeigten eine signifikante Abnahme der impfinduzierten humoralen Immunantwort drei und sechs Monate nach der Grundimmunisierung. Die Frequenzen von B-Gedächtniszellen und T-Zellen sind stabil geblieben oder gestiegen. Im Vergleich zu der Immunität nach der Grundimmunisierung war die humorale und zelluläre Immunität nach einer ersten Auffrischimpfung signifikant erhöht. Angesichts der Ungewissheit der Beschäftigten in den Unikliniken Deutschlands über die Notwendigkeit der Auffrischung des Impfschutzes (Scheithauer et al., 2022) war es zu diesem Zeitpunkt von entscheidender Bedeutung, die Wirksamkeit der Impfstoffe vor dem Höhepunkt der Omikron-Infektionswelle zu überprüfen (siehe Abb. 4, 7, 8 und 10). Im späteren Verlauf der COVID-19-Pandemie erwies sich die Booster-Impfung als die effektivste Primärpräventionsmaßnahme für beruflich exponierte Mitarbeiter des UKB, um das Auftreten einer schweren COVID-19-Erkrankung zu verhindern. Hinsichtlich der Wirksamkeit der mRNA-COVID-19-Impfstoffe gegen die derzeit zirkulierenden Omikron-Subvarianten kann auf der Grundlage der Daten zur kumulativen Inzidenz von SARS-CoV-2-Infektionsfällen aus der "Corona"-Abstrichambulanz des UKB (siehe **Tab. 4** und

Abb. 4) von einem anfänglich mäßigen Schutz gegen asymptomatische und milde Infektionen ausgegangen werden. Dies spiegelt sich in der Zunahme der Impfdurchbrüche am UKB zwischen Oktober 2021 und Januar 2023 wider. Diese Beobachtung entspricht der Dynamik von Immunreaktionen, die durch entsprechende Schutzimpfungen im Rahmen der Primärprävention bei anderen Viruserkrankungen hervorgerufen werden. Die meisten Immunantworten nehmen nach einer ersten und zweiten Auffrischimpfung oder einer ersten und zweiten Infektion im Laufe der Zeit ab (COVID-19 Forecasting Team-Stein et al., 2023). Eine vierte und fünfte Bestimmung der humoralen Immunantwort nach 18 bzw. 24 Monaten im Rahmen der „Moderna-Studie“ sowie in weiteren Publikationen (Davis-Gardner et al., 2023, Dewald et al., 2023, Hoehl et al., 2023, Marks et al., 2023, Yang et al., 2023, Barouch, 2022, Kaku et al., 2022) bestätigen diese Beobachtung.

1.4.3 Aktuelle Lage und Diskussion

Die am 09. November 2021 entdeckte SARS-CoV-2-Omikronvariante und die später im weiteren Verlauf entdeckten Subvarianten (BA.2, BA.4, BA.5, XBB.1-5, XAY, XBF) weisen geringere Hospitalisierungsraten nach einer Infektion auf und gehen aufgrund der Immunevasion durch die Mutationen mit einer signifikant höheren Infektiosität einher (Mannar et al., 2022). Nach den vorliegenden Daten des RKI vom 17.03.2022 blieb die Omikron-Variante in Deutschland die dominierende SARS-CoV-2-Variante. Initial wurde ein Anstieg des Gesamtanteils der rekombinanten Omikron-Variante XBB.1 registriert. Er lag in der Kalenderwoche (KW) 9/2023 bei 56 %. Der Anteil der rekombinanten XBF variierte zu diesem Zeitpunkt zwischen 1 % und 2 %. Die Rekombinante XAY wies ab der KW 5/2023 einen stabilen Anteil von knapp 1 % auf. Der Anteil der Omikron-Sublinien BA.5 und BA.2 ist in der Folgezeit weiter zurückgegangen. Der Anteil von BA.5 lag in der KW 9/2023 bei 24 %, der Anteil von BA.2 lag bei 16 % (Robert Koch-Institut, 2023). Seit Mai 2023 ist die Virusvariante XBB.1 und ihre Sublinien weltweit die dominierende Variante.

Eine Metaanalyse von Külper-Schiek et al., 2022 bei der 2,2 Millionen Probandinnen und Probanden berücksichtigt wurden, konnte zeigen, dass die Wirksamkeit der in der EU zugelassenen COVID-19-Impfstoffe bei der Omikron-Variante in Bezug auf die

Verhinderung einer SARS-CoV-2-Infektion nach vollständiger Grundimmunisierung gering und von kurzer Dauer ist. Yang et al., 2023 stellten fest, dass die Neutralisierung gegen die XBB-Linien, die durch die derzeitige Hybridimmunität nach einer Infektion mit BA.5 hervorgerufen wird, nach wie vor gering ist. Dies war ein Hinweis darauf, dass die Entwicklung der nächsten Generation von COVID-19-Impfstoffen auf der Basis von XBB-Sublinien und anderen zukünftigen Varianten weiterhin ein relevantes Thema war. Diese Publikationen validieren unsere Daten und unterstreichen die Bedeutung einer COVID-19-Auffrischimpfung im Rahmen der berufsspezifischen Exposition (Monzón-Posadas et al., Pušnik et al., 2023, Stein et al., 2023).

Diese Erkenntnis hat die Impfstoffhersteller in den letzten 24 Monaten dazu veranlasst mRNA-COVID-19-Impfstoffe zu weiterzuentwickeln. Inzwischen sind an die Omikron-Varianten angepasste bivalente Impfstoffe für den Markt zugelassen. Bedingt durch die genetische Variabilität von SARS-CoV-2 wurden COVID-19-Impfstoffe erneut an die aktuell zirkulierenden Virusvarianten (XBB.1.5) angepasst, um weiterhin einen möglichst optimalen Immunschutz gegen schwere COVID-19-Erkrankungen zu erreichen.
Diese Impfstoffe sollen die Übertragung von Infektionen mit Omikron-Subtypen wirksamer verhindern (Chalkias et al., 2022, Marks, 2023). Davis-Gardner et al., 2023 konnte zeigen, dass Personen, die den BA.5 enthaltenden bivalenten „Booster“ erhalten haben, eine bessere Neutralisierungsaktivität der Antikörper gegen alle Omikron-Subvarianten (insbesondere gegen BA.2.75.2, BQ.1.1 und XBB) aufwiesen als diejenigen, denen entweder ein oder zwei monovalente Booster verabreicht wurden.
Im Gegensatz dazu deuten Untersuchungen von Höhl et al. 2023 aus den USA zur Wirksamkeit der Omikron-adaptierten bivalenten mRNA-Impfstoffe (BA.1 und BA.4/5) auf eine ähnliche Schutzwirkung wie bei der Auffrischimpfung mit einem monovalenten Impfstoff hin. Gegenwärtig wird auch untersucht, ob eine frühere Impfung oder eine Infektion mit einer Prä-Omikron-Variante die adaptive Immunantwort auf die aktuell zirkulierenden Stämme möglicherweise beeinträchtigt. Das Argument der Antigenerbsünde oder Original Antigenic Sin (OAS), auch bekannt als Immune Imprinting, wird derzeit debattiert (Reynolds et al., 2022, Hoehl et al., 2023). Es ist nicht abschließend geklärt, ob die etwas höheren Antikörperkonzentrationen durch eine angepasste Vakzin-induzierte Immunität tatsächlich eine höhere Schutzwirkung im Hinblick auf alle VOC bedeuten. In diesem Zusammenhang zeigen unsere Daten (Pušnik et al. 2023), dass

Personen mit einer Hybridimmunität bisher eine besser korrelierte adaptive Immunantwort zeigen als „nur geimpfte Personen“.

Die $CD4^+$- und $CD8^+$-T-Zellen sowie die B-Gedächtnis-Zellen wurden bereits früher als eine besonders langlebige Komponente der zellulären Immunität identifiziert, die durch eine Infektion mit SARS-CoV-2 hervorgerufen wird (Monzón-Posadas et al., 2023, Pušnik et al., 2022, Terreri et al., 2022, Pušnik et al., 2023). Ähnlich wie eine Infektion löst auch eine Impfung den Aufbau von SARS-CoV-2-Spike-spezifischen Gedächtnis-B-Zellen aus (Goel et al., 2021). Die Anzahl dieser spezialisierten Zellen steigt jedoch noch mehrere Monate nach der Impfung an, im Gegensatz zu den Antikörpertitern (Terreri et al., 2022). Bei der aktuellen Diskussion über die Grenzen der Wirksamkeit von monovalenten und bivalenten Kombinationsimpfstoffen sollte dies berücksichtigt werden.

Die aktuelle Empfehlung der Weltgesundheitsorganisation (WHO) lautet, bei der notwendigen Anpassung des Impfstoffes als Impfantigen ein monovalentes Antigen zu verwenden, das von den SARS-CoV-2-Sublinien XBB.1.5 oder XBB.1.16 abgeleitet ist. Der erste an die XBB.1-Variante angepasste COVID-19-Impfstoff ist seit dem 18.09.2023 in Deutschland erhältlich (Comirnaty XBB.1.5). Die Marktzulassung für einen weiteren XBB.1.5-adaptierten mRNA-Impfstoff (Spikevax XBB.1.5) in der EU erfolgte am 15.09.2023. Für die nächsten Wochen ist die Zulassung eines proteinbasierten Impfstoffs auf der Basis von XBB.1.5 (Novavax) angekündigt. Entsprechend lautet die aktuelle Empfehlung der Ständigen Impfkommission am Robert Koch-Institut, besonders gefährdeten Personen und Risikogruppen weitere Auffrischimpfungen anzubieten. Eine zweite oder dritte Auffrischimpfung wird insbesondere auch immungesunden Beschäftigten des Gesundheitswesens angeboten, die im Rahmen ihrer Tätigkeit direkten Patientenkontakt haben.

1.4.4 Fazit

Die erfasste kumulative Inzidenz von SARS-CoV-2-Infektionen bei Probandinnen und Probanden der „Moderna-Studie“ am UKB im Zeitraum 01.03.2021 bis 28.02.2022 betrug 14%. Während der Studie wurde eine höhere Wirksamkeit der Impfstoffe gegen eine Infektion mit der Delta- im Vergleich zur Omikron-Variante festgestellt. Die mRNA-Impfstoffe bieten jedoch nach wie vor einen sehr guten Schutz gegen einen schweren

Verlauf der COVID-19-Erkrankung. Die gute Neutralisierungskraft gegenüber der Delta-Variante nach einer Booster-Impfung (siehe **Abb. 4, 5, 7, 8, 9 und 10**) deutet darauf hin, dass die niedrigere kumulative Inzidenz von SARS-CoV-2-Infektionen im Sommer 2021 trotz erhöhter berufsspezifischer Exposition mit dieser Variante nicht ausschließlich auf den saisonalen Charakter des Virus (Zeitfenster zwischen März und September 2021) zurückzuführen ist.

Die Limitation dieser Beobachtung liegt in der Kohorte und Anzahl der rekrutierten Probandinnen und Probanden (n = 77) für diese Studie. Diese Fallzahl wurde jedoch nach Rücksprache mit Statistikern des Instituts für Medizinische Biometrie, Informatik und Epidemiologie (IMBIE) der Universität Bonn bewusst im Studiendesign gewählt, um die knappen Ressourcen während der COVID-19-Pandemie am UKB möglichst effizient zu nutzen und statistisch signifikante Ergebnisse zu erzielen. Auf diese Weise wurde in Zeiten der Pandemie eine qualitativ hochwertige Analyse der Proben gewährleistet.

1.4.5 Ausblick

Antikörper sind im Blutplasma und anderen Körperflüssigkeiten einschließlich Speichel nachweisbar (Brandtzaeg, 2007). Diese Erkenntnisse werden bereits bei der Entwicklung bzw. Weiterentwicklung neuer Impfstoffe umgesetzt. SARS-CoV-2 infiziert zunächst die oberen Atemwege (V'kovski et al., 2021). Unsere Ergebnisse decken sich mit denen anderer Forscher und konnten zeigen, dass zwei Dosen des mRNA-Impfstoffs erfolgreich eine spike-spezifische Antikörperbildung im Speichel induzieren (Azzi et wo., 2022, Darwich et al., 2022, Monzón-Posadas et al., 2023). Ähnlich wie bei den im Plasma gefundenen Antikörpern nimmt ihre Konzentration im Laufe der Zeit rasch ab (Darwich et al., 2022), und kann durch eine Auffrischimpfung signifikant erhöht werden.

In der Entwicklung befindliche Impfstoffe, die nasal über die Schleimhaut verabreicht werden (IN-Impfstoffe), sind ein vielversprechender Ansatz zur Bekämpfung neuer Virusvarianten. Derzeit wird auch intensiv an verschiedenen IN-Impfstoffen gegen SARS-CoV-2 geforscht. Mindestens zwölf Impfstoffe befinden sich in unterschiedlichen Phasen der klinischen Prüfung (Alu et al., 2022).

Die neutralisierenden Antikörper sind im Allgemeinen der wichtigste antivirale Mechanismus, der durch die Impfung ausgelöst wird (Pollard et al., 2021). Die Bewertung

der SARS-CoV-2-Immunität stützt sich meist auf die Messung von spike-spezifischen Antikörpern im Blutplasma. Die in der “Moderna-Studie” gemessenen Spike-spezifischen Antikörperspiegel (als Biomarker) korrelierten für alle Varianten und Zeitpunkte mit der Plasmaneutralisationskapazität. Der Spike-spezifische Antikörpertiter (IgG) ist daher ein guter Surrogatparameter für die Neutralisation.

Eine Bestimmung des Antikörpertiters kann im Rahmen der Impfberatung auch bei Beschäftigten in Betracht gezogen werden, die gezielte Tätigkeiten im Sinne der Biostoffverordnung mit SARS-CoV-2 durchführen oder die im Rahmen der Patientenversorgung einer erhöhten berufsspezifischen Exposition ausgesetzt sind. Die Risikoabschätzung und die individuelle Bestimmung der Immunparameter spielen insbesondere bei schweren Impfunverträglichkeiten gegenüber mRNA-COVID-19-Impfstoffen eine entscheidende Rolle, um die Indikation für eine beruflich indizierte Schutzimpfung präziser zu stellen.

Aufgrund der genetischen Variabilität von SARS-CoV-2 und vor dem Hintergrund, dass die Impfstoff-induzierten Antikörpertiter mit der Zeit abnehmen, ist es weiterhin von großer Bedeutung, die Belastbarkeit der adaptiven Immunantwort nach COVID-19-Impfung oder SARS-CoV-2-Infektion im Hinblick auf die aktuell zirkulierenden Virusvarianten zu untersuchen. Dies kann wichtige Erkenntnisse für zukünftige Impf- und Auffrischungsstrategien liefern. Ziel ist es, auch in Zukunft einen möglichst optimalen Immunschutz des Personals in Einrichtungen der kritischen Infrastruktur und in Krankenhäusern der Maximalversorgung gegen eine schwere COVID-19-Erkrankung zu gewährleisten.

1.5 Zusammenfassung

Die COVID-19-Pandemie hatte enorme Auswirkungen auf die Bevölkerung in Deutschland. Im Rahmen der prospektiven infektionsepidemiologischen “Moderna-Studie” am UKB wurde die Wirksamkeit der adaptiven Immunantworten während einer zwölfmonatigen Zeitspanne (01.03.2021-28.02.2022) überprüft, sowohl nach Abschluss der Grundimmunisierung mit zwei Dosen eines mRNA-Impfstoffs als auch nach der ersten Auffrischungsdosis. Dazu wurden Blut- und Speichelproben von 77 Beschäftigten in Bezug auf die Immunantwort und die kumulative Inzidenz der SARS-CoV-2-Infektionen mit den dominanten VOC B.1.1.7 (Alpha), B.1.1.529 (Delta) und B.1.617.2 (Omikron) untersucht.

Zwischen dem dritten und sechsten Monat nach der Grundimmunisierung zeigte sich eine rasche Abnahme der Anti-Spike-IgG-Antikörper-Titer (1,7- bzw. 2,5-fache Abnahme in Plasma und Speichel; $p<0,0001$). Im Gegensatz dazu nahm die Konzentration der spike-spezifischen Gedächtnis-B-Zellen in diesem Zeitraum zu (2,4-facher Anstieg; $p<0,0001$), während die Konzentration der spike-spezifischen $CD4^+$- und $CD8^+$-T-Zellen für alle untersuchten Funktionen (Zytotoxizität, IFNγ-, IL-2- und TNFα-Expression) stabil blieb. Die Auffrischimpfung verbesserte die Antikörperantwort in Plasma und Speichel erheblich, mit signifikanten Veränderungen der Neutralisationskapazität gegenüber der derzeit zirkulierenden Omikron-Variante (25,6-facher Anstieg; $p<0,0001$). Der positive Effekt der Auffrischimpfung zeigte sich auch bei den spike-spezifischen IgG^+-Gedächtnis-B-Zellen (2,4-facher Anstieg; $p<0,0001$) und den zytotoxischen $CD4^+$- und $CD8^+$-T-Zellen (1,7- bzw. 1,9-facher Anstieg; $p<0,05$). Die kumulative Inzidenz der SARS-CoV-2-Infektionen über die Zeitspanne von zwölf Monaten betrug 0.14 (14 %).

Die ersten zugelassenen Impfstoffe, die auf Basis der neuen mRNA-Technologie entwickelt wurden, lösten insbesondere nach der ersten Auffrischungsimpfung eine starke Immunantwort aus. Im Rahmen dieser Studie war die Wirksamkeit der mRNA-Impfstoffe gegenüber einer SARS-CoV-2-Infektion mit der Alpha- oder Delta-Variante signifikant höher als gegenüber der Omikron-Variante. Gegen einen schweren Erkrankungsverlauf bieten diese Impfstoffe jedoch weiterhin bei allen drei Virusvarianten einen sehr guten Schutz.

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Longitudinal monitoring of mRNA-vaccine-induced immunity against SARS-CoV-2

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Background: Worldwide vaccination campaigns significantly reduced mortality caused by SARS-CoV-2 infection and diminished the devastating effects of the pandemic. The first approved vaccines are based on novel mRNA technology and elicit potent immune responses offering high levels of protection from severe disease.

Methods: Here we longitudinally assessed adaptive immune responses during a 12-month follow-up period after the initial immunization with 2 doses of mRNA vaccines and after the booster dose in blood and saliva.

Results: Our findings demonstrate a rapid waning of the anti-spike IgG titers between months 3 and 6 after the initial vaccination (1.7- and 2.5-fold decrease in plasma and saliva, respectively; P<0.0001). Conversely, the frequency of spike-specific memory B cells increased during this period (2.4-fold increase; P<0.0001) while the frequency of spike-specific CD4+ and CD8+ T cells remained stable for all assessed functions: cytotoxicity, IFNγ, IL-2, and TNFα expression. Booster vaccination significantly improved the antibody response in plasma and saliva, with the most profound changes observed in the neutralization capacity against the currently circulating omicron variant (25.6-fold increase; P<0.0001). The positive effect of booster vaccination was also evident for spike-specific IgG+ memory B cell (2.4-fold increase; P<0.0001) and cytotoxic CD4+ and CD8+ T cell responses (1.7- and 1.9-fold increase respectively; P<0.05).

Conclusions: Collectively, our findings offer a detailed insight into the kinetics of adaptive immune response following SARS-CoV-2 vaccination and underline the beneficial effects of a booster vaccination.

KEYWORDS

SARS-CoV-2, COVID-19, B cell, T cell, antibody, vaccination, booster, longitudinal

Introduction

The vaccination campaign against the SARS-CoV-2 infection was launched at the beginning of 2021 with the hope to dampen the devastating effects of pandemics by reducing transmission and mortality caused by COVID-19. Remarkably, the first two vaccines to receive approval for use were based on a novel mRNA technology that had not been previously applied in any of the marketed vaccines.

The BNT162b2 and mRNA-1273 vaccines demonstrated an outstanding efficacy of over 90% reduction in severe COVID-19 cases and proved safe for use (1, 2). The high efficacy was due to the robust immune response elicited by the 2-dose full vaccination regimen (1–4). Several studies have confirmed that mRNA vaccines induce the production of neutralizing antibodies, with titers well above those induced by the natural infection (4–6). Furthermore, the mRNA vaccines were effective at triggering the formation of memory B cell and T cell responses (7, 8). These features of mRNA vaccines raised high hopes for the worldwide SARS-CoV-2 vaccination campaign to curb the ongoing pandemic. Unfortunately, however, the virus mutated more rapidly and new variants emerged that would overcome vaccine-induced immunity (9–12). The wild-type virus was consecutively succeeded by the alpha, delta, and omicron variants accumulating mutations in the spike protein (13). The currently circulating omicron variant has more than 30 amino-acid changes in the spike protein, compared to alpha and delta variants where typically less than 15 amino-acid are observed (14). This substantial increase in the antigenic distance from the wild-type spike of vaccines leads to efficient immune evasion and higher transmission rates even in countries with high vaccination rates or natural immunity (15). Furthermore, mutations influenced the replication biology of the variants. Particularly, changes near the furin-cleavage site have been suggested to be associated with enhanced cell entry and increased transmissibility (16, 17).

In addition, it has soon become clear that the immune response induced by vaccines, although initially very potent, rapidly wanes with time after vaccination opening a window of opportunity for breakthrough infections (18). Numerous studies have demonstrated the declining antibody titers with time after the initial vaccination and increased rates of breakthrough infections (13, 19, 20) making it evident that the 2-dose vaccination regimen will not be sufficient for long-term protection from COVID-19 and that booster vaccinations will be necessary to keep immunity at high levels (18, 21). At the beginning of fall 2021, the third dose of mRNA vaccines was approved and recommended firstly for high-risk groups and subsequently for the general population. The booster vaccination initially restored vaccine efficacy by triggering a potent recall immune response (22), but similarly waned rather rapidly (23).

As of November 2022, the vaccine coverage in Germany is 78% for at least one dose, 76% for two, and 62% for three vaccine doses (24). Given that the majority of the population has been vaccinated and that the vaccine-induced antibody titers decline with time, it is important to investigate the persistence of the adaptive immune response following vaccination as this may give critical insights into future vaccination and booster strategies.

Here we performed a comprehensive longitudinal assessment of the adaptive immune response to the initial two doses but also a booster dose of mRNA vaccine in healthy SARS-CoV-2 naïve individuals. Our findings demonstrate the waning of the immune response following vaccination and emphasize the beneficial effect of a booster vaccination.

Materials and methods

Study cohort

A total of 77 individuals that were initially vaccinated with 2 doses of mRNA-based SARS-CoV-2 vaccine and 9 months later received a booster vaccination were recruited for the study. All individuals were SARS-CoV-2 naïve at the beginning of the study. Breakthrough infections were monitored by RT-PCR and anti-nucleocapsid ELISA as a part of routine screening at the diagnostics department of the Institute of Virology, University Hospital Bonn. Individuals that contracted an infection were excluded from the findings described in this paper. All participants were either employed or studied at the University of Bonn at the time of sampling but were not necessarily healthcare workers.

Ethics approval

All participants provided written informed consent approved by the Ethics Committee of the Medical Faculty of the University of Bonn (ethics approval numbers 125/21).

Sample collection and storage

Study participants provided peripheral blood specimens, saliva, and pharyngeal swabs. Blood was centrifuged and EDTA-plasma was stored until analysis (-80°C). Before the saliva collection participants were instructed not to eat or drink for at least 60 min. Participants were than advised to retain saliva for 1-2 min and expectorate it in a centrifuge tube. Saliva samples were centrifuged to remove solid particles and frozen at -20°C. PBMC were isolated by density gradient centrifugation and cryopreserved in liquid nitrogen.

2 Determination of SARS-CoV-S1-specific IgG in plasma

S1-specific IgG titers were determined using an in-house quantitative ELISA. Therefore, microtiter plates with high binding capacity were coated with 100 µl of coating buffer (carbonate-bicarbonate buffer, pH=9.6) containing 1 µg/ml of recombinant S1 domain of the SARS-CoV-2 spike protein (Biotinylated SARS-CoV-2 (COVID-19) S1 protein, Acrobiolabs). Plates were subsequently sealed and incubated overnight at 4°C. Coated plates were washed with wash buffer (PBS with 0.05% (v/v) Tween®-20) and blocked (PBS containing 1% (w/v) BSA) to prevent unspecific binding. Cryopreserved EDTA plasma samples were thawed at room temperature and diluted at previously optimized dilution of 1:3200

in a blocking buffer. After blocking plates were washed, incubated with plasma samples and standards (serially diluted pooled plasma of vaccinated individuals), washed again, and incubated with 100 µl HRP-conjugated anti-IgG antibody (Goat anti-Human IgG (Heavy chain) Secondary Antibody, HRP, Invitrogen) diluted 1:8000 in wash buffer. If not stated differently, incubation steps were performed for 1 hour at 37°C. Finally, plates were washed and 100 µl of the substrate solution (TMB Chromogen Solution, Life technologies) was added. The reaction developed at room temperature for 5 min until the addition of 50 µl of 0.2 M H_2SO_4. Optical density at 450 nm was measured immediately after the reaction was stopped. The background-subtracted OD_{450} readings were interpolated onto the standard dilution curve that had previously been calibrated to the international WHO standard (NIBSC reference number: 20/136). To determine the positivity cutoff we measured plasma samples from 30 individuals that have never been exposed to SARS-CoV-2. Based on the measurements we determined the cutoff (mean+2xSD) from which the results were considered positive for anti-S1 IgG; 19.2 BAU/mL.

Determination of SARS-CoV-2 S1-specific IgG and IgA in saliva

The titers of S1-specific IgA and IgG in saliva were measured by in-house quantitative ELISA. Therefore, high-binding microtiter plates were coated with 100 µl of coating buffer (carbonate-bicarbonate buffer, pH=9.6) containing 1 µg/ml of recombinant SARS-CoV-2 S1 protein (Biotinylated SARS-CoV-2 (COVID-19) S1 protein, Acrobiolabs). Following overnight incubation at 4°C, the plates were washed (PBS with 0.05% (v/v) Tween®-20), blocked (PBS containing 3% (w/v) BSA), and washed again. Saliva samples were thawed, diluted 1:16 in sample buffer (PBS containing 1% (w/v) BSA), and applied onto S1-coated plates. Blocking and incubation with saliva samples and standard dilutions were performed at 37°C for 1 hour. Subsequently, plates were washed and incubated at 37°C for 1 hour with 100 µl HRP-conjugated anti-IgG antibody (Goat anti-Human IgG (Heavy chain) Secondary Antibody, HRP, Invitrogen) diluted 1:8000 in wash buffer or 100 µl HRP-conjugated anti-IgA antibody (Goat anti-Human IgA (Heavy chain) Secondary Antibody, HRP, Invitrogen) diluted 1:1000 in wash buffer. After the incubation with secondary antibodies, plates were washed and 100 µl of the substrate (TMB ELISA Substrate, High Sensitivity, Abcam) was added. The reaction was stopped by the addition of 100 µl of 1 M H_2SO_4 after developing for 5 min at room temperature. Optical density at 450 nm was measured immediately after the addition of the stop solution. The background-subtracted OD_{450} readings were interpolated to the standard dilution curve, derived from measurements of serially diluted highly positive saliva samples, to obtain concentration units relative to the standard (arbitrary units indicated as a.u.). These units are not comparable to those of the plasma ELISA. To determine the positivity cutoff we measured saliva samples from 24 individuals that were seronegative for anti- SARS-CoV-2-spike IgG. Based on the measurements we determined the cutoff (mean+2xSD) from which the results were considered positive; 0.014 a.u. for IgG and 0.012 a.u. for IgA. The same concentrations of S1-specific monoclonal antibody (anti-SARS-CoV-2-RBD antibody, clone CR3022, Abcam) with IgA or IgG constant region was measured to make the OD_{450} readings comparable between the IgG and IgA assays.

Plaque reduction neutralization assay

The plasma neutralization capacity was determined by a plaque reduction neutralization assay as previously described (25). Briefly, plasma was heat-inactivated and serially two-fold diluted starting with 2-fold up to 32768-fold dilution. Each dilution was combined with 80 plaque-forming units of SARS-CoV-2 (either wild-type, delta, or omicron variant). The inoculum was then added to Vero E6 cells. After the incubation, the inoculum was removed, and cells were overlaid with carboxymethylcellulose-containing media. After 3 days, plates were fixed and stained with crystal violet solution revealing the formation of plaques. The number of plaques was plotted against the serum dilutions, and IC_{50} was determined using the GraphPad Prism software.

Immunomagnetic isolation of B cells

B cells were enriched from cryopreserved PBMC samples by positive immunomagnetic isolation (Human CD19 MultiSort Kit, Miltenyi Biotec) following the manufacturer's instructions. Briefly, thawed, and rested PBMCs were resuspended in recommended isolation buffer and labeled with anti-CD19 antibodies coupled to magnetic beads. Bead-labeled cells were then immobilized onto a magnetic column. The column was washed and the flow-through containing B-cell-depleted PBMC was set aside for the assessment of T-cell responses. The column was removed from the magnetic field and immobilized B cells were washed out. To remove the magnetic beads and anti-CD19 antibodies, B cells were treated with enzymes disintegrating the immunomagnetic complexes.

Detection of S1-specific memory B cells by flow cytometry

Antigen-specific B cells were identified by immunofluorescent tagging with recombinant wild-type SARS-CoV-2 S1 protein, as previously described (25). Briefly, the cells were incubated with the recombinant S1 protein conjugated to two different fluorophores, stained for viability, and subsequently incubated with a mixture of fluorescently labeled antibodies binding surface antigens. Labeled cells were acquired on a flow cytometer (BD FACS Celesta). The frequency of S-specific memory B cells was calculated by subtracting the average frequency of S1-binding memory B cells in healthy donor samples collected before the outbreak of SARS-CoV-2.

Ex vivo stimulation of T cells

B-cell-depleted PBMC fractions were seeded in 96-well U bottom plates and stimulated with wild-type SARS-CoV-2 PepTivator (Miltenyi Biotec) overlapping peptide pools spanning the entire

sequence of spike (S) protein, in presence of anti-CD107a-APC (clone H4A3; Biolegend) antibody. One million cells were stimulated per condition, and the final concentration of each peptide in the stimulation mix was 1 μg/ml. As a co-stimulatory signal, antibodies binding CD28 and CD49d (BD FastImmune^{TM} CD28/CD49d) were added to a final concentration of 1 μg/ml. Stimulation was performed at 37°C for a total of 6 hours. As a negative control, an equally treated DMSO-stimulated sample was included for each biological replicate. As positive control cells stimulated with PMA (20 ng/ml) and ionomycin (1 μg/ml) were used. One hour into stimulation, Golgi Stop and Golgi Plug (BD Bioscience) were added (final concentration 1 μg/ml) to inhibit vesicular transport and prevent the secretion of the cytokines from cells.

Detection of SARS-CoV-2-specific T cells by flow cytometry

Stimulated cells were washed with PBS, and stained with Zombie Aqua (Biolegend) dye for 15 min at 4°C to discriminate viable cells. Subsequently, samples were washed with FACS buffer (PBS supplemented with 2% FCS, 2 mM EDTA, and 0.05% NaN_3), fixed, and permeabilized in CytoFix/CytoPerm Solution (BD Bioscience) for 15 min at 4°C. Cells were then washed with 1x Perm/Wash Buffer (BD Bioscience), and stained for intracellular markers for 15 min at 4°C using the following antibody conjugates; anti-CD3-APC-Cy7 (clone UCHT1; Biolegend), anti-CD4-BV786 (clone SK3; BD Bioscience), anti-IFNγ-PE (clone B27; Biolegend), anti-TNFα-BV421 (clone Mab11; Biolegend), and anti-IL2-AF488 (clone MQ1-17H12; Biolegend). Labeled cells were then washed with PBS and acquired on FACS Celesta (BD Bioscience). Frequencies of antigen-specific CD4+ T cells were calculated as negative-control-subtracted data. Possible longitudinal fluctuations in laser intensity were monitored and adjusted before each experiment using fluorescent beads (Rainbow beads, Biolegend). The data were analyzed with the FlowJo Software version 10.0.7 (TreeStar).

Statistical analysis

Statistical analysis was performed using R software (26). Differences between the groups were assessed using the Wilcoxon test for matched data with Holm's correction for multiple testing. The strength of correlations was evaluated by Spearman's test. Statistical significance is indicated by the following annotations: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Results

Study design

To assess the longevity of the adaptive immune response following SARS-CoV-2 vaccination we monitored antibody, memory B cell, and memory T cell levels of 50 vaccinated individuals for 12 months after the full vaccination with two doses of mRNA vaccine. Samples were taken 3, 6, and 12 months after full vaccination. All individuals received booster vaccination about 9 months after the initial immunization. Individuals with breakthrough infections were excluded from the analysis. Additionally, a group of 20 individuals was recruited to follow the dynamics of immune response immediately after booster vaccination. These individuals were sampled 3 and 15 weeks post-immunization and received the 3 vaccine doses at the same time as the other 50 study participants (Figure 1A).

Vaccination-induced antibodies wane over time

Neutralizing antibodies are the primary antiviral mechanism induced by vaccination and the best-defined correlate of protection against SARS-CoV-2 infection (27). We, therefore, measured plasma levels of IgG specific for the S1 subunit of the SARS-CoV-2 spike protein using an in-house ELISA calibrated to the international WHO standard (NIBSC reference number: 20/136). Our findings indicate that the level of S1-specific IgG declines by 1.7-fold between months 3 and 6 after vaccination with 2 doses of mRNA vaccine (P<0.0001) and then increases by 3.4-fold following booster vaccination between months 6 and 12 (P<0.0001) (Figure 1B). Between weeks 3 and 15 after the booster shot, the level of S1-specific IgG decreased 2.5-fold (P<0.0001) (Figure 1C). To assess the dynamics of exclusively neutralizing antibodies, we next performed plaque reduction neutralization assays using live un-manipulated SARS-CoV-2 isolates (wild-type, delta, and omicron variants). The neutralization capacity against the wild-type virus declined 5-fold during months 3 and 6 (P<0.0001). In the case of delta and omicron variants, the reductions in neutralizing potency were 2.5- and 5-fold respectively (P<0.0001, P<0.05). On month 12, roughly 3 months following the booster vaccination, the neutralization levels rose 7.6-fold, 6.3-fold, and 25.6-fold for wild-type, delta, and omicron respectively when compared to month 6 (P<0.0001, P<0.0001, P<0.0001) (Figure 1D). Furthermore, we observed a 3.3-fold decrease in neutralization capacity against the wild-type virus (P<0.0001), a 2-fold decrease in neutralization capacity against the delta (P<0.01), and a 2.5-fold decrease in neutralization capacity against the omicron variant (P<0.001) between weeks 3 and 15 after the booster vaccination (Figure 1E). To demonstrate the different susceptibilities of SARS-CoV-2 variants to neutralization, we next directly compared the plasma neutralization capacity of wild-type, delta, and omicron variants at months 3, 6, and 12 after full 2-dose vaccination. At the first time point, the ancestral variant was most susceptible to neutralization, followed by the delta variant, while omicron showed to be notably more resistant. Interestingly, at month 6, the plasma of vaccinated individuals more efficiently neutralized the delta variant than the wild-type. Omicron remained the most resistant to neutralization at all time points, however, its susceptibility to neutralization considerably increased after booster vaccination (Figure 1F). We next compared the ratio between the plasma neutralization capacity and S1-specific IgG titers for all three

variants and time points. The data suggest a decreased proportion of neutralizing antibodies against all three variants for month 6 when compared to months 3 and 12. In the case of delta and omicron variants, the highest proportion of neutralizing antibodies was observed after the booster vaccination on month 12 (Figure 1G). High S1-specific IgG titers translated well into higher neutralization capacity since we observed strong correlations between the two parameters for all three variants and time points. The correlations were slightly weaker in the case of the omicron variant (Figure 1H).

Taken together, our findings demonstrate a waning of the initial antibody response against SARS-CoV-2 after the 2 initial doses but also after the third booster shot of the mRNA vaccine. Importantly, booster vaccination improved the potency of antibody response against the SARS-CoV-2 variants compared to 2 vaccine doses only.

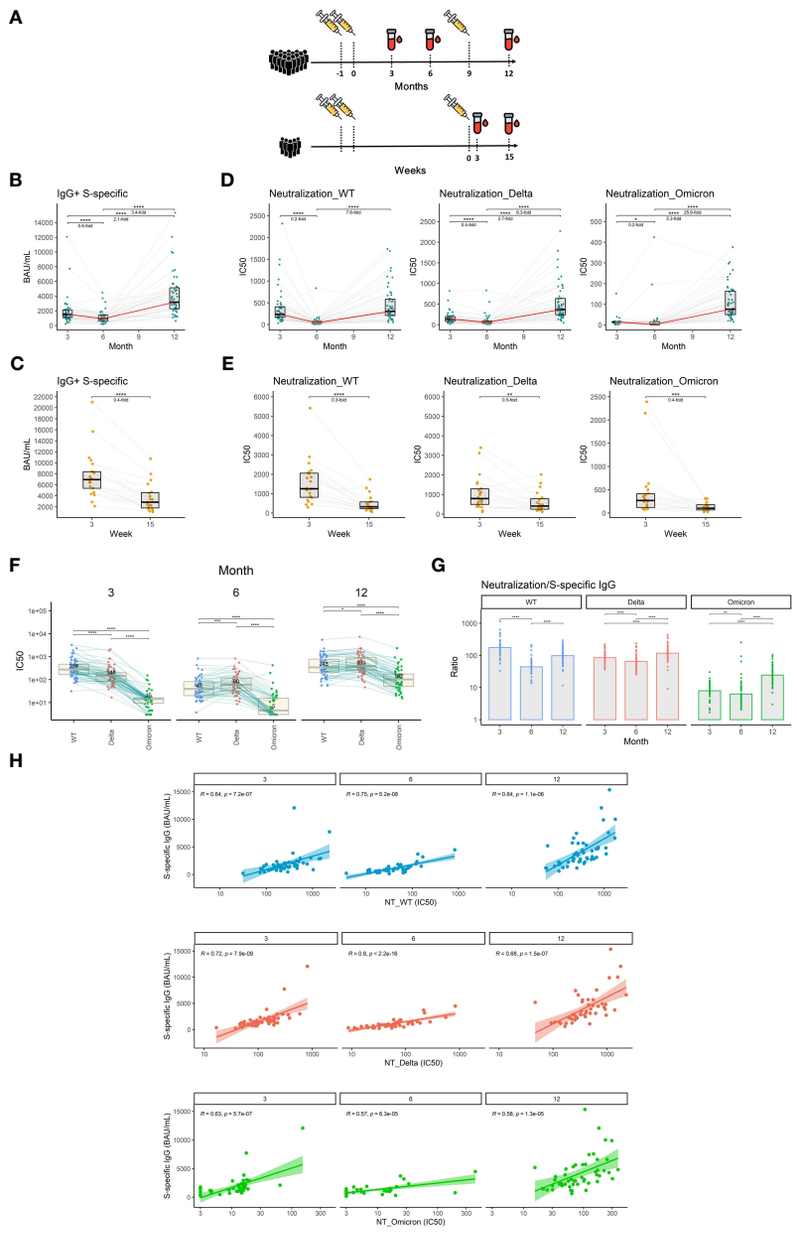

FIGURE 1
The dynamics of S1-specific antibody response in plasma after the initial and booster doses of mRNA vaccine. **(A)** Timelines demonstrating temporal relationships between vaccination and sampling events of the study participants. **(B)** S1-specific IgG levels in international units (BAU/mL) measured at different time points after the initial vaccination. **(C)** S1-specific IgG levels in international units measured 3 and 15 weeks after the booster vaccination. **(D)** Plasma neutralization capacity measured against the wild-type, delta, and omicron variants for months 3, 6, and 12 after the initial vaccination. **(E)** Plasma neutralization capacity measured against the wild-type, delta, and omicron variants for weeks 3 and 15 after the booster vaccination. The red line connects the median values of each time point. Fold change was calculated as a ratio of the medians of compared time points. **(F)** Comparison of neutralization susceptibility of SARS-CoV-2 variants for months 3, 6, and 12. Median values are given within the boxplots. **(G)** Ratio between the neutralization capacity and S1-specific IgG titers for different time points and different SARS-CoV-2 variants. **(H)** Correlation between plasma levels of S1-specific IgG and plasma neutralization capacity for wild-type, delta, and omicron variants. Differences between the groups were assessed using the Wilcoxon test for matched data. Correction for multiple testing was performed using Holm's method. The strength of correlations was assessed by Spearman's correlation test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

Booster vaccination augments SARS-CoV-2-specific antibody levels in saliva

As a respiratory virus, SARS-CoV-2 initially infects the upper respiratory tract (28) and infection might be prevented by the presence of antibodies in the upper mucosa, mucus, and saliva.

We, therefore, measured the titers of S1-specific IgG and IgA with an ultrasensitive ELISA in saliva. Our findings demonstrate that S1-specific IgG levels in saliva decrease by 2.5-fold between months 3 and 6 after full vaccination (P<0.0001). Following booster vaccination in month 9, the S1-specific IgG levels increased 8.4-fold on month 12 when compared to month 6 (P<0.0001) (Figure 2A). In contrast, the S1-specific IgA titer remained relatively stable during the entire monitoring with a subtle 1.3-fold increase between months 6 and 12 (P<0.001) (Figure 2B). Since we used the same monoclonal antibody with a variable Fc region as a calibrator for the IgG and IgA ELISAs, we were able to compare the relative amounts of both antibody isotypes. Interestingly, the proportion of S1-binding IgA was significantly increased at month 6 when compared to month 3 (P<0.05), suggesting that IgA persists longer in saliva than IgG. At month 12 IgG was the predominant isotype of salivary antibodies recognizing the S1-domain of the spike protein. Its proportion was significantly higher than at months 3 and 6 (P<0.01 and P<0.0001, respectively) (Figure 2C). The levels of S1-specific IgA and IgG in saliva correlated for all time points (Figure 2D), as did the levels of S1-specific IgG in saliva and plasma (Figure 2E).

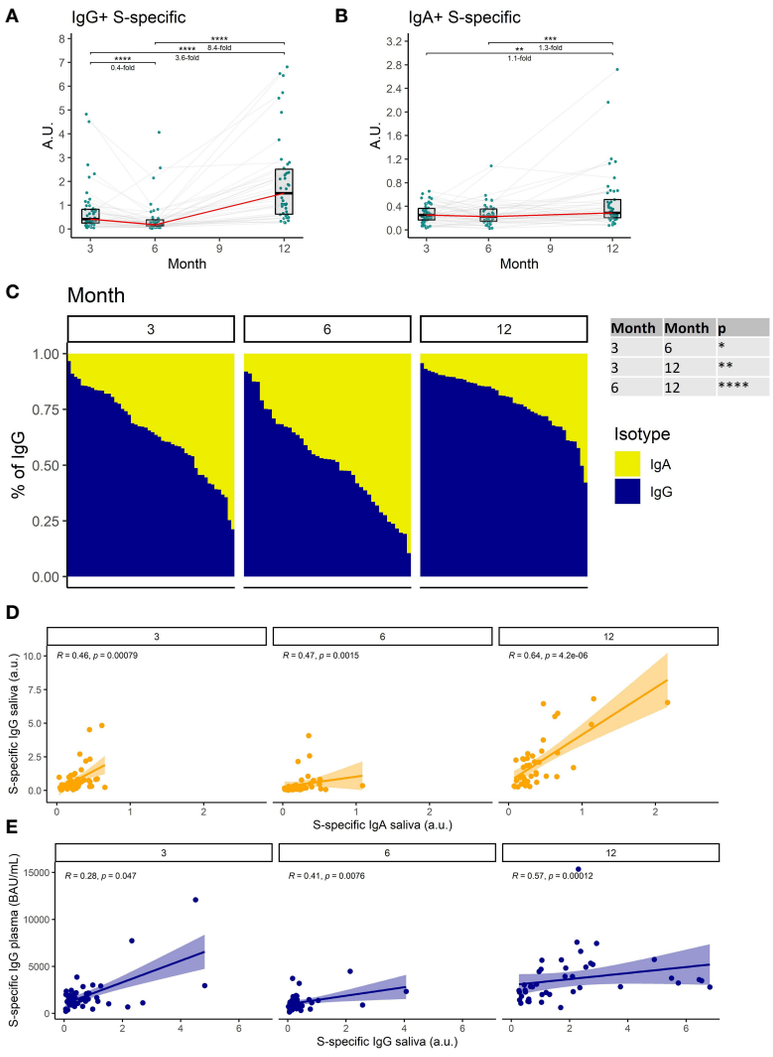

FIGURE 2
The dynamics of S1-specific antibody response in saliva after the initial and booster doses of mRNA vaccine. S1-specific **(A)** IgG, **(B)** IgA, levels in the saliva of vaccinated individuals for months 3, 6, and 12 after the initial vaccination. The red line connects the median values of each time point. Fold change was calculated as a ratio of the medians of compared time points. **(C)** Relative proportions of IgA and IgG isotypes among the S1-specific antibodies in the saliva of vaccinated individuals. **(D)** Correlations between the salivary S1-specific IgG and IgA for different time points. **(E)** Correlations between the plasma and salivary S1-specific IgG for different time points. Differences between the groups were assessed using the Wilcoxon test with Holm's correction for multiple testing. The strength of correlations was assessed by Spearman's correlation test. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Collectively, these data demonstrate a decrease of SARS-CoV-2-specific antibodies in saliva with time after full vaccination and the resurge of mostly IgG antibodies following booster vaccination.

The frequency of SARS-CoV-2-specific memory B cells increases over time and with booster vaccination

Studies have shown that SARS-CoV-2-specific memory B cells remain at elevated levels for a much longer time after the infection or vaccination than the antibodies making them particularly important for long-term immunological memory (25, 29).

We, therefore, measured the frequency of S1-specific memory B cells in the peripheral blood of vaccinated individuals at each of the sampling time points utilizing multiparameter flow cytometry. We distinguished S1-specific memory B cells according to their B cell receptor (BCR) isotype; IgA+, IgM+, IgG+ (Figure 3A) (detailed gating strategy is available in Supplemental figure 1). Unlike in the case of antibodies the frequency of IgG+ S1-specific memory B cells increased between months 3 and 6 by 1.6-fold (P<0.001) and further rose 2.4-fold (P<0.0001) between months 6 and 12 (Figure 3B). The frequency of these cells only decreased immediately following the recall response evoked by the booster vaccination; 2-fold decrease between weeks 3 and 15 following the booster (P<0.05) (Figure 3C). The frequencies of IgA+ and IgM+ S1-specific memory B cells

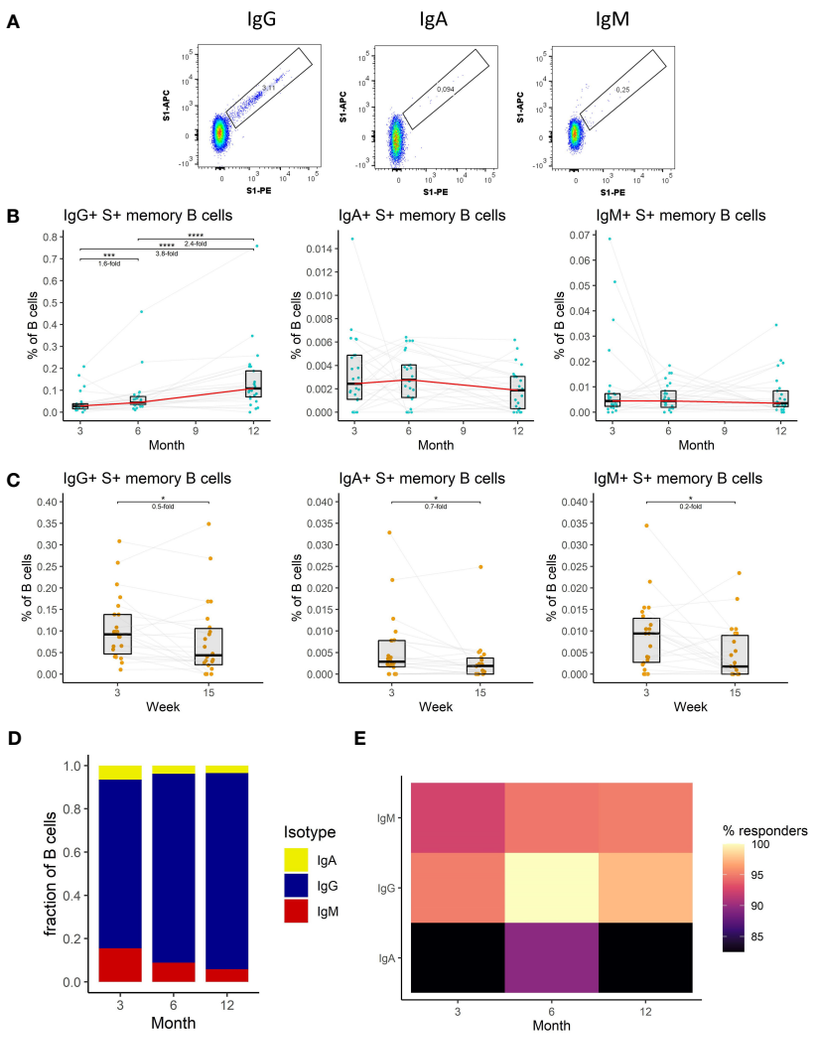

FIGURE 3
The dynamics of SARS-CoV-2-specific memory B cell response after the initial and booster doses of mRNA vaccine. **(A)** Representative flow cytometry plots for identification of S1-specific memory B cells with different BCRs. **(B)** Frequencies of IgG+, IgA+, and IgM+ S1-specific memory B cells as a percentage of total B cells in the peripheral blood of vaccinated individuals for months 3, 6, and 12 after the initial vaccination. The red line connects the median values of each time point. Fold change was calculated as a ratio of the medians of compared time points. **(C)** Frequencies of IgG+, IgA+, and IgM + S1-specific memory B cells in peripheral blood of vaccinated individuals 3 to 15 weeks after the booster vaccination. **(D)** Relative proportions of S1-specific memory B cells bearing BCRs of a different isotype. **(E)** Percentage of individuals with detectable S1-specific memory B cells according to the BCR isotype and time point. Differences between the groups were assessed using the Wilcoxon test with Holm's correction for multiple testing.

remained stable between months 3, 6, and 12 (Figure 3B), but also decreased between the third and fifteenth week following the booster vaccination (1.4-fold, P<0.05, and 5-fold, P<0.05 respectively) (Figure 3C). Next, we compared the relative frequencies of S1-specific memory B cells according to their BCR isotype for months 3, 6, and 12 after the full vaccination. Of note, the frequencies of S1-specific IgA+ and IgM+ memory B cells were much lower than those of IgG+ cells. Their proportions were highest at month 3 and then decreased with each of the following time points (Figure 3D). The percentages of individuals with detectable S1-specific memory B cells were generally high. Most of the individuals had detectable IgG+ S1-specific memory B cells followed by IgM+, individuals with IgA+ cells were rare (Figure 3E).

To sum up, we have shown that IgG+ memory B cells dominate the SARS-CoV-2-specific B cell response. The frequency of these cells kept increasing with time. Following the booster vaccination, their frequency initially dropped but remained elevated compared to the initial 2-dose immunization.

SARS-CoV-2-specific CD4+ T cell responses are durable and moderately augmented by booster vaccination

CD4+ T cells are a key component of a vaccine-induced immune response since they regulate antibody production by B cells (30, 31). Besides their stimulatory and coordinating functions, these cells can also act cytotoxic and directly kill infected cells (32).

Given their importance, we measured the frequencies of CD4+ T cells specific for the spike (S) protein of the SARS-CoV-2 in the peripheral blood of vaccinated individuals. Antigen-specific T cells were detected by peptide stimulation and subsequent detection of cytokine expression by multiparameter flow cytometry. Four major functions of the CD4+ T cells were monitored: cytotoxicity (CD107a and IFNγ expression), IFNγ expression, IL-2 expression, and TNFα expression (Figure 4A) (detailed gating strategy is available in Supplemental figure 2). The frequency of S-specific CD4+ T cells remained stable between months 3 and 6 after the full vaccination regardless of the function. After the booster vaccination on month 9, the frequency of S-specific cytotoxic CD4+ T cells rose by 1.7-fold (P<0.05), and the frequency of IFNγ-expressing CD4+ T cells rose by 1.6-fold (P<0.05) between the months 6 and 12. Frequencies of IL-2- and TNFα-expressing CD4+ T cells did not significantly change during this period (Figure 4B). Between weeks 3 and 15 following booster vaccination we observed a decline in S-specific CD4+ T cell frequencies. The frequency of cytotoxic cells decreased 3.3-fold (P<0.0001), the frequency of IFNγ-expressing cells 2-fold (P<0.01), the frequency of IL-2-expressing cells 1.7-fold (P<0.05) and the frequency of TNFα-expressing cells 1.7-fold (P<0.05) (Figure 4C). TNFα expression was the most frequent function among the S-specific CD4+ T cells followed by IL-2 expression, IFNγ expression, and cytotoxicity. This was true for months 3, 6, and 12 (Figure 4D).

Collectively, these findings indicate that vaccination induces a durable SARS-CoV-2-specific CD4+ T cell response that is moderately augmented by the booster vaccination.

SARS-CoV-2-specific CD8+ T cell responses are durable and moderately augmented by booster vaccination

CD8+ T cells can recognize and kill infected cells and thus represent an important antiviral mechanism. Studies have shown that SARS-CoV-2-specific CD8+ T cells successfully limit the infection and positively correlate with protection from severe disease (33, 34).

We, therefore, investigated S-specific CD8+ T cell responses in the peripheral blood of vaccinated individuals. Antigen-specific cells were identified by peptide stimulation and flow-cytometric detection of effector molecules (Figure 5A) (detailed gating strategy is available in Supplemental figure 2) as in the case of CD4+ T cells. The data revealed increased frequencies of cytotoxic and TNFα-expressing S-specific CD8+ T cells at month 12 after vaccination. The frequency of cytotoxic cells increased by 1.9-fold (P<0.05) between months 3 and 12, while the frequency of TNFα-expressing cells increased 1.3-fold (P<0.05) during the same period. No significant changes were observed between months 3 and 6 for any of the assessed functions (Figure 5B). Unlike the rest of the immune responses, the frequency of S-specific CD8+ T cells did not decline between weeks 3 and 15 after the booster vaccination, for any of the functions, suggesting the persistence of these cells (Figure 5C). The frequencies of cytotoxic, IFNγ- and TNFα-expressing S-specific CD8+ T cells did not change over time, while the frequency of IL2-expressing cells trended to be lower (Figure 5D). Comparing CD8+ and CD4+ S-specific T cell responses, CD4+ T cells were generally more frequent than CD8+ T cells. The highest proportion of CD8+ T cells was observed among the cytotoxic followed by IFNγ-expressing, TNFα-expressing, and IL2-expressing S-specific T cells. No significant differences were observed between the three time points for any of the functions (Figure 5E). Similarly, the percentage of individuals with detectable S-specific T cells was higher in the case of CD4+ T cells for all functions and at all three time points (Figure 5F).

Taken together, we have demonstrated that mRNA vaccines induce SARS-CoV-2-specific CD8+ T cells that remain stable after initial and booster vaccinations. These cells were considerably less frequent than SARS-CoV-2-specific CD4+ T cells.

Discussion

There is increasing evidence that SARS-CoV-2 vaccination mounts a robust adaptive immune response (1–4), however, similar to infection-induced immunity, the longevity of this response is limited (35, 36). In addition to the ongoing emergence of new variants, this is one of the primary reasons behind the increasing infection incidence despite the high seropositivity of the population. Here we longitudinally assessed the adaptive immune response to the initial 2 doses but also the third booster dose of mRNA vaccine in healthy SARS-CoV-2 naïve individuals. Our data demonstrate a decline in the antibody response after the initial vaccination. At the same time, memory B cell and T cell frequencies proved to be more stable or even increased in magnitude. Importantly, booster

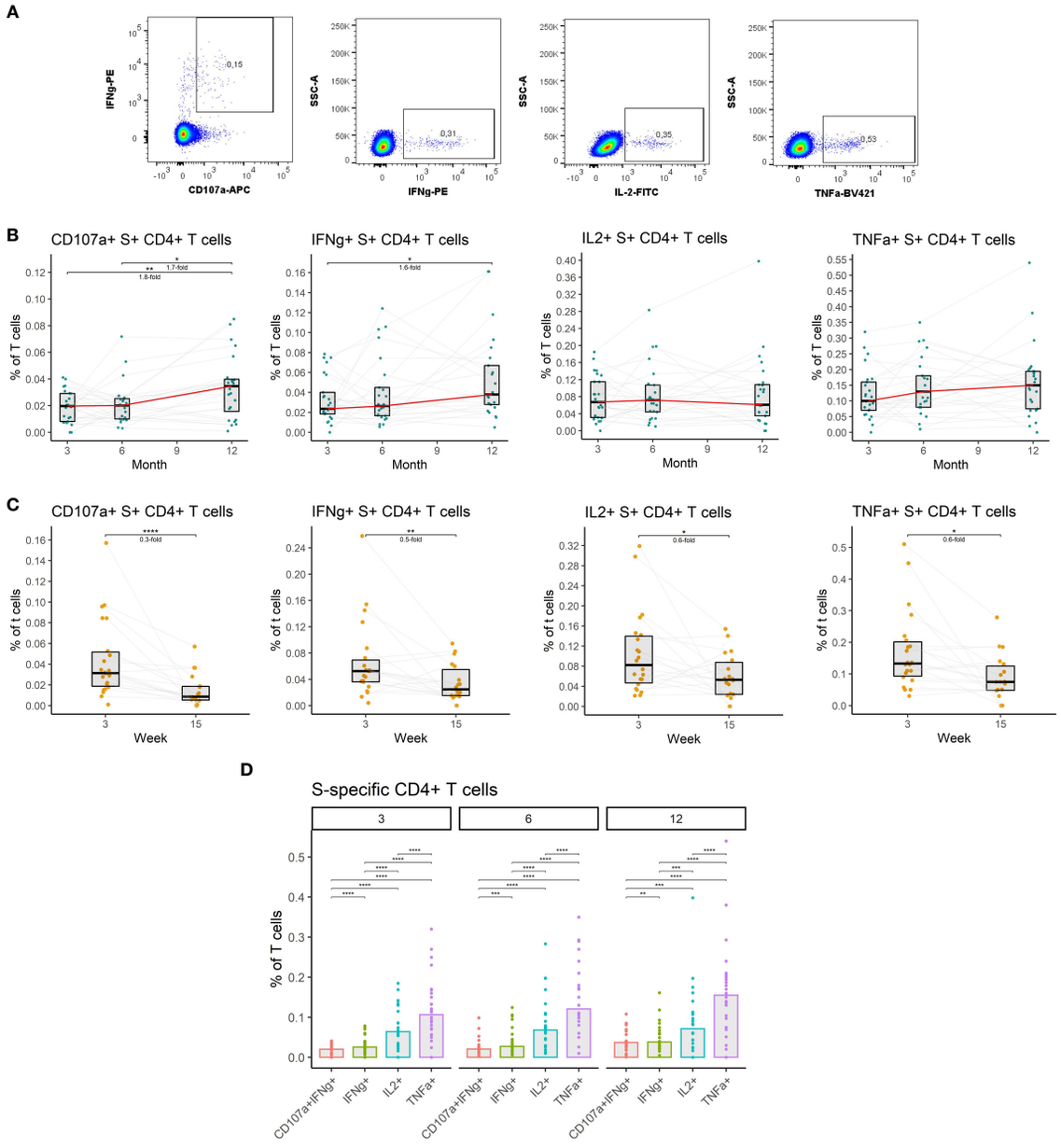

FIGURE 4
The dynamics of SARS-CoV-2-specific CD4+ T cells after the initial and booster doses of mRNA vaccine. **(A)** Representative flow cytometry plots demonstrating the detection of S-specific CD4+ T cells with different effector functions; cytotoxicity (CD107a and IFNγ expression), IFNγ expression, IL-2 expression, and TNFα expression. **(B)** The frequencies of S-specific CD4+ T cells with different effector functions as a percentage of bulk T cells for months 3, 6, and 12 after the initial vaccination. The red line connects the median values of each time point. Fold change was calculated as a ratio of the medians of compared time points. **(C)** The frequencies of S-specific CD4+ T cells with different effector functions in the peripheral blood of vaccinated individuals 3 to 15 weeks after the booster vaccination. **(D)** Comparison of S-specific CD4+ T cell frequencies with different functions for months 3, 6, and 12 after the initial vaccination. Differences between the groups were assessed using the Wilcoxon test with Holm's correction for multiple testing. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

vaccination significantly improved humoral and cellular responses compared to the initial two doses of mRNA vaccine. Nevertheless, most immune responses decreased with time after the booster dose.

Neutralizing antibodies are generally the main antiviral mechanism induced by vaccination (37) and the assessment of SARS-CoV-2 immunity most often relies on the measurement of spike-specific antibodies in plasma. In concordance with previous studies (35, 36, 38), we have demonstrated that the neutralizing antibody titer rapidly declines after the initial immunization with 2 doses of mRNA vaccine as well as after the booster vaccination. The booster vaccination remarkably augmented the neutralizing antibody levels when compared to the initial vaccination (39). Of note, the most profound boosting effect was observed for the omicron variant which has generally been more resistant to neutralization than alpha and delta variants. Apart from increasing the overall spike-specific and neutralizing antibody titer booster dose also increased the

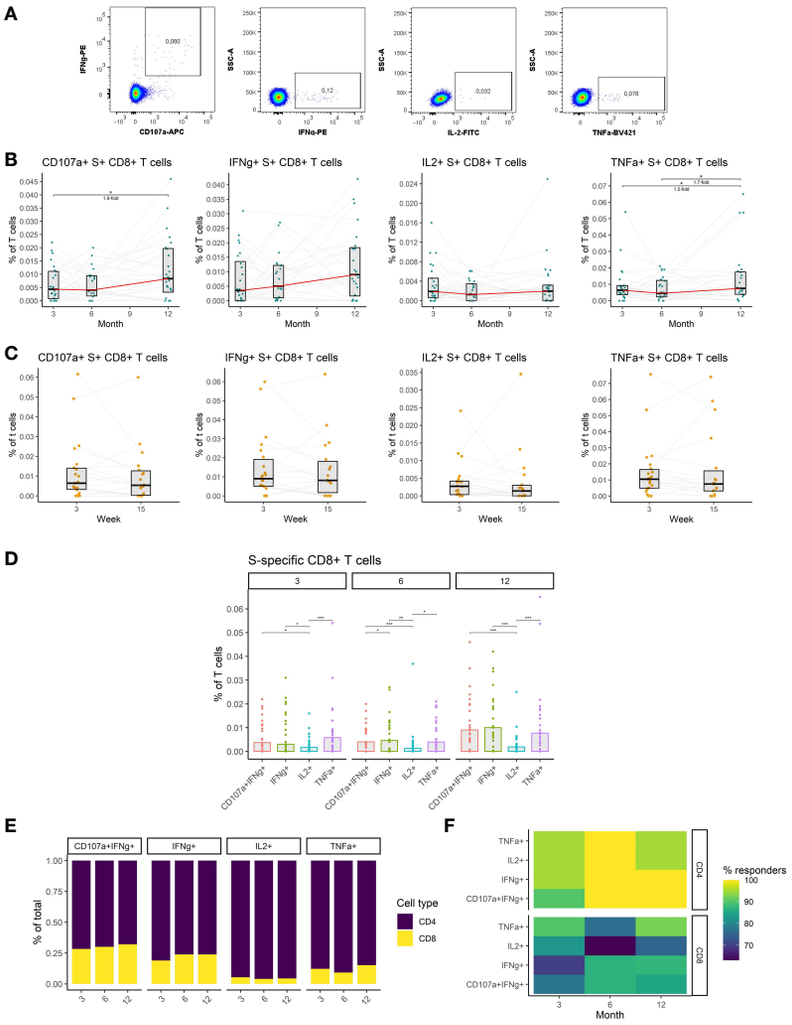

FIGURE 5
The dynamics of SARS-CoV-2-specific CD8+ T cells after the initial and booster doses of mRNA vaccine. **(A)** Representative flow cytometry plots demonstrating the detection of S-specific CD8+ T cells with different effector functions; cytotoxicity (CD107a and IFNγ expression), IFNγ expression, IL-2 expression, and TNFα expression. **(B)** The frequencies of S-specific CD8+ T cells with different effector functions as a percentage of bulk T cells for months 3, 6, and 12 after the initial vaccination. The red line connects the median values of each time point. Fold change was calculated as a ratio of the medians of compared time points. **(C)** The frequencies of S-specific CD8+ T cells with different effector functions in the peripheral blood of vaccinated individuals 3 to 15 weeks after the booster vaccination. **(D)** Comparison of S-specific CD8+ T cell frequencies with different functions for months 3, 6, and 12 after the initial vaccination. **(E)** Relative proportions of CD4+ and CD8+ S-specific T cells with different effector functions for months 3, 6, and 12 after the initial vaccination. **(F)** Percentage of individuals with detectable S1-specific CD4+ or CD8+ T cells according to the effector function and time point. Differences between the groups were assessed using the Wilcoxon test with Holm's correction for multiple testing. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

neutralizing potency of these antibodies against the delta and omicron variants. Improved potency and breath of SARS-CoV-2-neutralizing antibodies after booster vaccination has also been previously observed and has been attributed to the increased proportion of B cell clones targeting conserved regions of the receptor-binding domain (40, 41). Furthermore, spike-specific antibody levels correlated with plasma neutralization capacity for all variants and time points indicating that spike-specific antibody titer is a good surrogate of neutralization. Interestingly, the correlations at 6 months after vaccination showed the best association between the antibody titer and neutralization suggesting maturation of the antibody response with time after vaccination. Collectively, these findings demonstrate a rapid waning of antibody response and emphasize the importance of booster vaccination.

Apart from plasma, antibodies can also be found in other body fluids including saliva (42). This might be particularly important since SARS-CoV-2 initially infects the upper respiratory tract (28). Our findings and those of others show that 2 doses of mRNA vaccine successfully elicited spike-specific antibodies in saliva (43, 44). Similar to those found in plasma, their titer rapidly declined with time as previously documented (43), and got augmented by booster vaccination. Interestingly, IgA antibodies showed higher stability over time than IgG but were less abundant, especially early after the vaccination. The levels of both isotypes correlated for all time points

indicating coordinated production. Moreover, the levels of spike-specific IgG in saliva correlated with the plasma levels of these antibodies for all time points, suggesting that salivary anti-spike IgG partially originate from the plasma (42). To sum up, antibodies in saliva generally followed the same kinetics as those in plasma, however, IgA showed increased stability and might be important for long-term protection from infection.

Memory B cells have previously been identified as a particularly persistent component of immunity elicited by SARS-CoV-2 infection (25, 29). Similar to infection, also vaccination triggers the buildup of SARS-CoV-2 spike-specific memory B cells (7, 45). In contrast to antibodies, however, their frequencies increase for several months after vaccination (29). A similar trend was observed for spike-specific IgG+ but not IgM+ and IgA+ memory B cells following the initial two doses of mRNA vaccine. While there was a decline in the frequency of spike-specific memory B cells immediately after booster vaccination, which is probably due to the downregulation of the immune response following antigen clearance, their levels increased compared to the initial vaccination, as previously reported by others (46). IgG was the predominant BCR isotype for all time points as previously observed for individuals recovered from infection (25), and its proportion kept increasing over time. Although much less frequent than IgG, IgM-bearing spike-specific memory B cells were more abundant than those with IgA BCR. Moreover, spike-specific IgA+ memory B cells were not detectable in the largest proportion of individuals for all time points. Collectively, IgG+ spike-specific memory B cell frequencies increase for at least 6 months after the initial vaccination and further expand after the booster vaccination making them a key component of long-term SARS-CoV-2 immunity.

To efficiently generate an antibody response a vaccine must also trigger the formation of virus-specific CD4+ T cells that provide B cells with signals crucial for the production of high-affinity antibodies. Studies have demonstrated that mRNA vaccines successfully induce CD4+ T cell responses (8, 30). We have demonstrated that the frequencies of spike-specific CD4+ T cells with different functions (cytotoxicity, IFNγ expression, IL-2 expression, and TNFα expression) remained relatively stable for at least 6 months after the initial 2 doses of vaccine. Similar to B cells, their frequencies rapidly declined between 3 and 15 weeks following booster vaccination. Compared to the initial vaccination, the booster dose moderately increased frequencies of cytotoxic and IFNγ-expressing cells but did not affect the cells with other functions as previously observed (47). TNFα expression was the most prevalent function among the spike-specific CD4+ T cells followed by IL-2 expression, IFNγ expression, and cytotoxicity. Taken together these findings suggest the persistence of SARS-CoV-2 spike-specific CD4+ T cells following initial vaccination and the moderate effect of booster vaccination on these cells.

It has been previously documented that mRNA vaccines induce the formation of CD8+ T cell response (48–50). Similar to CD4+ T cells, we have observed that CD8+ T cell frequencies remain relatively stable after the initial immunization. In contrast to other immune responses, no decrease in the frequency of these cells was observed between weeks 3 and 15 following booster vaccination. In line with previous studies booster dose only moderately increased the frequencies of cytotoxic and TNFα-expressing cells compared to the initial immunization (48). Most of the spike-specific CD8+ T cells were either cytotoxic, expressed IFNγ or TNFα. IL-2-expressing cells were rare. The observed differences in the functional profiles of CD4+ and CD8+ T cells are most likely due to the different biologies of the two cell kinds; CD8 T cells are less capable of IL-2 production than CD4s (51). Considering the ratio between CD4+ and CD8+ T cells among the spike-specific T cells, the latter were notably less abundant for all time points. Furthermore, a larger proportion of individuals lacked spike-specific CD8+ than CD4+ T cell response. This can be explained by the design of mRNA vaccines that primarily targets the production of antibodies and not cytotoxic T cells. Altogether, SARS-CoV-2–specific T cell frequencies were stable after the initial 2-dose vaccination and mildly increased after the booster vaccination. Of note, CD4+ T cells were more abundant than CD8+ T cells.

In the present study, we have assessed the adaptive immune response to the mRNA vaccines after the initial and booster immunizations in healthy SARS-CoV-2 naïve individuals. We showed that antibody titer decreases rapidly after the initial 2 doses but is augmented following the booster vaccination. Booster vaccination was particularly important for the neutralization of the currently circulating omicron variant. Similar kinetics were observed for the salivary antibodies, with exception of IgA whose levels were relatively stable. The memory B cells and T cells showed to be more durable than the antibodies and were also positively affected by booster vaccination, making them particularly important for durable protection against severe SARS-CoV-2 infection.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Medical Faculty of the University of Bonn (ethics approval numbers 125/21). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

Conceptualization, JP, WP. Methodology, JP. Investigation, JP, WP, KP, JZ, MB, CS, HP. Resources, JP, WP. Writing-Original Draft, JP, WP. Writing-Review & Editing, HS. Funding acquisition, JP, HS. Supervision, JP, HS. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare no competing interests. The idea, the plan, the concept, the protocol, the conduct, the data analysis, and the writing of the manuscript of this study were independent of any third parties, including the funding agency.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2023.1066123/full#supplementary-material

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SARS-CoV-2 humoral and cellular immunity following different combinations of vaccination and breakthrough infection

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The elicited anti-SARS-CoV-2 immunity is becoming increasingly complex with individuals receiving a different number of vaccine doses paired with or without recovery from breakthrough infections with different variants. Here we analyze the immunity of individuals that initially received two doses of mRNA vaccine and either received a booster vaccination, recovered from a breakthrough infection, or both. Our data suggest that two vaccine doses and delta breakthrough infection or three vaccine doses and optionally omicron or delta infection provide better B cell immunity than the initial two doses of mRNA vaccine with or without alpha breakthrough infection. A particularly potent B cell response against the currently circulating omicron variant (B.1.1.529) was observed for thrice vaccinated individuals with omicron breakthrough infection; a 46-fold increase in plasma neutralization compared to two vaccine doses ($p < 0.0001$). The T cell response after two vaccine doses is not significantly influenced by additional antigen exposures. Of note, individuals with hybrid immunity show better correlated adaptive immune responses compared to those only vaccinated. Taken together, our data provide a detailed insight into SARS-CoV-2 immunity following different antigen exposure scenarios.

The worldwide vaccination campaign against SARS-CoV-2 infections demonstrated outstanding results in cutting down the severity of infections[1–3]. The first approved vaccines were based on the novel mRNA technology and triggered a robust immune response resulting in high protection efficiency[4–7]. This was reflected in the low frequency of breakthrough infections among the vaccinated, which were mostly caused by the alpha variant (Pango lineage B.1.1.7), and resulted in mild disease[8,9]. However, similar to infection-induced immunity, the immune response to SARS-CoV-2 vaccines rapidly declines below the level required for protection from infection[10,11]. By the time the first vaccines were rolled out, the SARS-CoV-2 pandemic was already established in most of the populated regions on earth[12]. With 100,000 new infections per week globally[12], the virus had plenty of space to mutate and adapt in a way that would overcome the immune protection of the vaccinated population. The waning immunity and the emergence of new viral variants like delta (Pango lineage B.1.617.2) fuelled the increasing frequency of breakthrough infections[13,14]. Although severe Covid-19 cases were less frequent in previously vaccinated than unvaccinated individuals it became clear that booster vaccinations will be needed to maintain protection from severe SARS-CoV-2 infection[15,16]. In developed countries like Germany, the majority of the vaccinated individuals received a third vaccine dose significantly

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improving the immune response[17,18]. Recently, a fourth vaccine dose was recommended for individuals at high risk for severe disease. However, in particular, against the currently circulating omicron variant (Pango lineage B. 1.1.529) the vaccine-induced immunity offers little protection from infection as seen in high rates of breakthrough infections[12]. Protection from severe diseases nevertheless remains high among vaccinated individuals[19].

SARS-CoV-2 infection of vaccinated individuals rarely develops a severe disease course, and most of the infections resolve without life-threatening consequences[14]. Importantly, these breakthrough infections further strengthen the immunity established by vaccination. Studies have shown that vaccinated individuals that also recovered from a SARS-CoV-2 infection have comparable neutralizing antibody titers to those that received three vaccine doses, and are therefore better protected from the severe disease than twice-vaccinated individuals[20–23]. Furthermore, breakthrough infections compensate for the waning of immunity established by previous infection or vaccination[20]. However, there is heterogeneity in breakthrough infections amongst vaccinated individuals in terms of the total number of antigen exposures (defined as an immune response to infection or vaccination throughout the manuscript) and the variant that causes the infection. We postulated that individuals that initially received two doses of mRNA vaccine and later became infected with different variants and/or received a third vaccine dose have distinct immunity profiles depending on the antigen exposure. In this observational study, we compared the antibody levels, B cell, and T cell responses amongst the individuals belonging to seven different groups based on their antigen exposure history: 2-times vaccinated (2xVacc), 2-times vaccinated followed by alpha breakthrough infection (2xVacc+α), 2-times vaccinated followed by delta breakthrough (2xVacc+δ), 3-times vaccinated (3xVacc), 3-times vaccinated followed by omicron infection (3xVacc+o), 3-times vaccinated where the third dose was preceded by an alpha infection (3xVacc+α), and 3-times vaccinated where the third dose was preceded by a delta infection (3xVacc+δ).

Results

Not only the number but also the type of antigen exposure is important for potent humoral immunity against the SARS-CoV-2

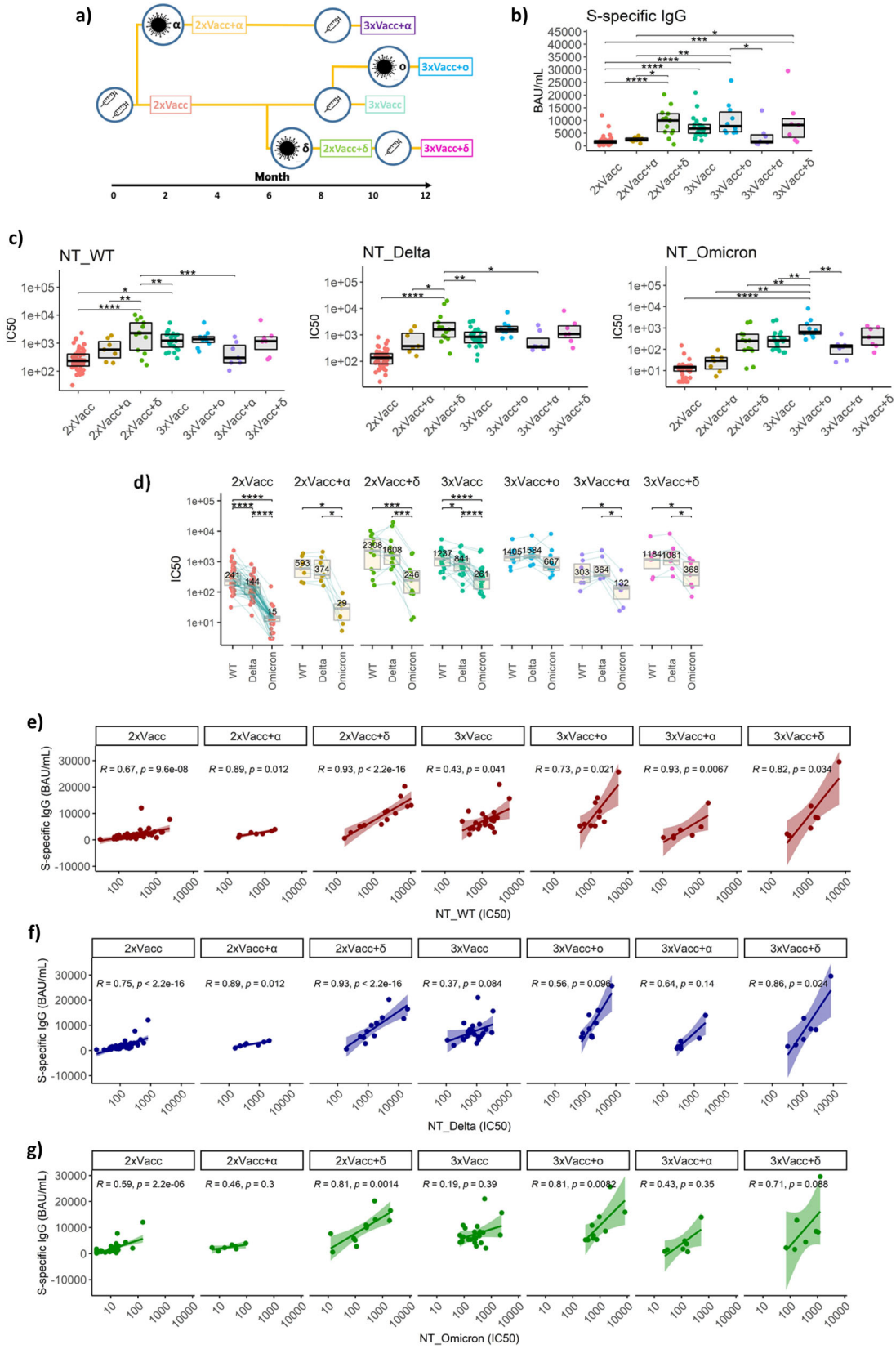
Antibodies are the best-defined correlate of protection against the SARS-CoV-2 infection[24], therefore, we first investigated levels of neutralizing antibodies among individuals with different antigen exposure histories. Based on the antigen exposure status we defined the following groups: 2-times vaccinated (2xVacc, $n = 54$), 2-times vaccinated followed by alpha breakthrough infection (2xVacc+α, $n = 7$), 2-times vaccinated followed by delta breakthrough (2xVacc+δ, $n = 13$), 3-times vaccinated (3xVacc, $n = 23$), 3-times vaccinated followed by omicron infection (3xVacc+o, $n = 10$), 3-times vaccinated where the third dose was preceded by an alpha infection (3xVacc+α, $n = 7$), and 3-times vaccinated where the third dose was preceded by a delta infection (3xVacc+δ, $n = 7$) (Fig. 1a). The detailed information on the antigen exposure and sampling time points along with the demographic information is provided in the supplement (Supplemental Fig. 1 and Supplemental Table 1).

Determination of plasma IgG titer against the S1 subunit of the SARS-CoV-2 spike protein was carried out by the in-house ELISA calibrated to the international WHO standard (NIBSC reference number: 20/136). The data revealed that 2xVacc+δ, 3xVacc, 3xVacc+o, and 3xVacc+δ groups had significantly higher S1-specific IgG titers compared to the 2xVacc group and the groups with an alpha breakthrough infection; 2xVacc+α, 3xVacc+α (Fig. 1b). The largest difference (5.2-fold, $p < 0.0001$) was observed between the 2xVacc+δ and 2xVacc groups. To investigate whether the same pattern can be observed for only neutralizing antibodies we performed plaque reduction assays using live unmanipulated SARS-CoV-2 isolates: wild-type (Pango lineage A), delta, and omicron variants. We observed similar differences between the groups as for the S1-binding IgG measured by ELISA for all variants. Of note, the 2xVacc+δ group showed particularly high neutralization potency against the wild-type (6.5-fold compared to 2xVacc, $p < 0.0001$) and delta variants (7.8-fold compared to 2xVacc, $p < 0.0001$), while the 3xVacc+o group was the most efficient at neutralizing the omicron variant (46-fold compared to 2xVacc, $p < 0.0001$) (Fig. 1c). Comparing the three SARS-CoV-2 variants and their susceptibility to neutralization we observed that the omicron variant was significantly more resistant to neutralization than delta and wild-type. The most profound reduction in the neutralization capacity against the omicron variant was observed for the 2xVacc and 2xVacc+α groups (16-fold and 20-fold respectively when compared to the wild-type). The only exception was the 3xVacc+o group that equally neutralized all three variants. Importantly, delta showed significantly higher resistance to neutralization than the wild-type exclusively in the case of the groups without breakthrough infections; 2xVacc, 3xVacc (Fig. 1d). To assess the relationship between the S1-specific IgG levels and neutralization capacity against the wild-type virus we correlated the two parameters for each of the antigen exposure groups. The level of S1-binding antibodies correlated with neutralization regardless of the antigen exposure history. The strongest correlations were observed in case of the 2xVacc+α ($r = 0.89$, $p = 0.012$), 3xVacc+α ($r = 0.93$, $p = 0.0067$), 2xVacc+δ ($r = 0.93$, $p < 0.0001$), and 3xVacc+δ ($r = 0.82$, $p = 0.034$) groups (Fig. 1e). Moreover, we correlated the S1-specific IgG titers with neutralization of the delta variant. The groups with the strongest correlations were in this case 2xVacc+δ ($r = 0.93$, $p < 0.0001$), 3xVacc+δ ($r = 0.86$, $p = 0.024$), and 2xVacc+α ($r = 0.89$, $p = 0.012$) (Fig. 1f). Correlations with neutralization of the omicron variant were notably weaker compared to the other two variants and significant only for the 3 out of 7 groups; 2xVacc, 2xVacc+δ, and 3xVacc+o. Relatively strong associations were observed only for the 2xVacc+δ ($r = 0.81$, $p = 0.0014$) and 3xVacc+o ($r = 0.81$, $p = 0.0082$) groups (Fig. 1f). Furthermore, we assessed the presence of antibodies specific for the nucleocapsid (N) protein of the SARS-CoV-2 using a commercial Roche Cobass assay. Complying with findings for the S1-specific IgG levels we observed that 77% of the delta, and 100% of omicron, but only 43% of the alpha breakthrough infections led to seroconversion.

Taken together our findings demonstrate that two vaccine doses and delta breakthrough infection or three vaccine doses and optionally omicron or delta infection provide significantly better humoral immunity to SARS-CoV-2 infection compared to the baseline antigen exposure with 2 doses of mRNA vaccine. A particularly strong humoral response against different SARS-CoV-2 variants was observed among twice-vaccinated individuals that got infected with the delta variant and thrice-vaccinated individuals that recovered from omicron breakthrough infection.

Salivary S1-specific antibody levels are highly dependent on the antigen exposure scenario

SARS-CoV-2 initially replicates in the upper respiratory tract where it is exposed to antibodies present in mucosa and saliva[25]. Therefore, the SARS-CoV-2-specific antibody titer in saliva might be a better correlate of protection from infection than the level of antibodies in plasma[26,27]. To determine the titer of IgG and IgA specific for the S1 subunit of the SARS-CoV-2 spike protein we developed an ELISA detecting comparatively low amounts of antibodies present in saliva. We demonstrated that similar to S1-specific plasma IgG levels, 2xVacc+δ, 3xVacc, 3xVacc+o, and 3xVacc+δ groups had significantly higher IgG levels compared to the baseline 2xVacc group, while the groups with an alpha breakthrough infection; 2xVacc+α, 3xVacc+α had relatively few S-specific IgG in saliva. Particularly high S1-specific IgG levels were observed in the 3xVacc+o group which had a 13-fold higher titer than the baseline 2xVacc group ($p < 0.0001$) (Fig. 2a). In contrast to IgG, salivary S1-specific IgA levels were only significantly increased in the



case of 2xVacc+δ and 3xVacc+o groups (Fig. 2b). Since we used the same monoclonal antibody with variable Fc region as a calibrator for the IgG and IgA ELISAs we were able to compare the relative binding strengths of the two antibody isotypes. The highest cumulative binding strength of S1-specific IgG and IgA was observed for the 3xVacc+o group (a 9.5-fold increase compared to 2xVacc; $p < 0.0001$) followed by 2xVacc+δ, 3xVacc, and 3xVacc+δ. 2xVacc and 2xVacc+α groups were significantly lower than most of the other groups (Fig. 2c). Regardless of the antigen exposure group, IgG showed a stronger binding capacity than IgA. The highest proportion of IgA binding was observed for the 2xVacc group and the lowest for the 3xVacc group (Fig. 2d). We next investigated whether S1-specific IgG levels in saliva reflect those measured in plasma. In contrast to plasma, the only significant correlations in saliva were observed for the 2xVacc ($r = 0.28$,

Fig. 1 | Plasma antibody response after different combinations of vaccination and breakthrough infection. a Schematic chronological representation of antigen exposures defining the seven groups compared in this study. Detailed information about the antigen exposure and sampling time points is provided as supplemental material. **b** S1-specific IgG levels in international units measured for the seven groups with different antigen exposure histories. Exact *p* values in sequential order from the upmost bracket: $^{*}p = 0.028$, $^{***}p = 0.00021$, $^{*}p = 0.032$, $^{**}p < 0.0038$, $^{****}p < 0.0001$, $^{****}p < 0.0001$, $^{*}p = 0.011$, $^{****}p < 0.0001$. **c** Plasma neutralization capacity against wild-type, delta, and omicron variants. Exact *p* values in sequential order from the upmost bracket of the left graph: $^{***}p = 0.00071$, $^{**}p = 0.0027$, $^{*}p = 0.021$, $^{**}p = 0.0023$, $^{****}p < 0.0001$, $^{*}p = 0.04$, $^{**}p = 0.0034$, $^{*}p = 0.04$, $^{****}p < 0.0001$, $^{**}p = 0.0035$, $^{**}p = 0.0018$, $^{**}p = 0.0081$, $^{**}p = 0.0011$, $^{****}p < 0.0001$. **d** Comparison of neutralization susceptibility of SARS-CoV-2 variants for each of the analyzed groups. The IC50 medians are given numerically for each boxplot. Exact p values in sequential order from the upmost bracket of the leftmost group: $^{****}p < 0.0001$, $^{****}p < 0.0001$, $^{****}p < 0.0001$, $^{*}p = 0.047$, $^{*}p = 0.047$, $^{***}p = 0.00073$, $^{***}p = 0.00073$, $^{****}p < 0.0001$, $^{*}p = 0.023$, $^{****}p < 0.0001$, $^{*}p = 0.047$, $^{*}p = 0.047$, $^{*}p = 0.047$, $^{*}p = 0.047$. In panels **b**–**d** the data is displayed as boxplots, indicating the first quartile, median, and third quartile, with individual data points. Correlation between plasma levels of S1-specific IgG and plasma neutralization capacity for **e** wild-type, **f** delta, **g** omicron variants. The 95% confidence intervals around the line of best fit are displayed as shading. The *r* and *p* values are given for each line. The following numbers of biologically independent samples were included in each group for all the graphs in this figure: 2xVacc, $n = 54$; 2xVacc+α, $n = 7$; 2xVacc+δ, $n = 13$; 3xVacc, $n = 23$; 3xVacc+o, $n = 10$; 3xVacc+α, $n = 7$; 3xVacc+δ, $n = 7$. Differences between the groups were assessed using the Mann–Whitney test or Wilcoxon test for matched data. The strength of correlations was assessed by Spearman's correlation test. Correction for multiple testing was performed using Holm's method, all statistical tests were two-sided. Source data are provided as a Source data file.

$p = 0.044$), 2xVacc+α ($r = 0.93$, $p = 0.0067$), and 2xVacc+δ ($r = 0.75$, $p = 0.012$) groups (Fig. 2e).

Collectively, we have shown that the individuals that received 3 vaccine doses and afterward acquired an omicron infection show the highest S1-specific antibody titer in saliva. Interestingly, IgG represented the majority of S1-binding antibodies in saliva and its levels correlated with S1-specific plasma IgG only in cases of particular antigen exposure combinations.

The frequency of S1-specific memory B cells is influenced by the antigen exposure history

When assessing the quality of SARS-CoV-2 immunity it is important to not only consider the humoral but also cellular components that are particularly important for long-term protection from the disease[28].

We, therefore, measured the frequency of S1-specific memory B cells in the peripheral blood of individuals with different antigen exposure histories utilizing multiparameter flow-cytometry (Fig. 3a). Detailed gating strategy for identification of S1-specific memory B cells is provided in Supplemental Fig. 2. Our data demonstrate increased frequencies of IgG+ S1-specific memory B cells for 2xVacc+δ, 3xVacc, and 3xVacc+o groups when compared to the 2xVacc and 2xVacc+α groups. In the case of the 3xVacc+δ and 3xVacc+α groups, the frequencies of these cells were relatively low and comparable to the baseline 2xVacc group (Fig. 3b). No statistically significant differences between the antigen exposure groups were observed for the IgA+ and IgM+ S1-specific memory B cells (Fig. 3c, d, respectively). When considering the frequency of the total S1-specific memory B cells, regardless of the B cell receptor (BCR) isotype, the 3xVacc+o group had the highest frequency of these cells (a 3.5-fold increase compared to the 2xVacc group; $p < 0.05$) followed by 2xVacc+δ and 3xVacc groups. The frequencies observed within 3xVacc+δ, 3xVacc+α, and 2xVacc+α groups were comparable to the 2xVacc group (Fig. 3e). We next investigated the relative proportions of S1-specific memory B cells with different BCR isotypes. We observed that IgG+ S1-specific memory B cells were the most frequent for all groups compared ($p < 0.001$). 2xVacc and 2xVacc+α groups had significantly increased levels of non-IgG+ S1-specific memory B cells compared to 2xVacc+δ and 3xVacc+δ groups ($p < 0.05$ for all comparisons) (Fig. 3f).

Taken together our findings demonstrate increased frequencies of S1-specific memory B cells among individuals that either received 3 doses of mRNA vaccine, 3 doses of mRNA vaccine plus omicron infection, or 2 doses of vaccine and delta infection. IgG was the most prevalent BCR isotype of S1-specific memory B cells regardless of the antigen exposure history.

Different antigen exposure combinations do not significantly affect SARS-CoV-2-specific T cells

T cells contribute to the defense against viral infections by coordinating the production of antibodies and killing the infected cells. It has been previously shown that T cells specific for SARS-CoV-2 successfully limit the infection and positively correlate with protection from severe disease[29,30].

Given their importance we next measured the frequencies of CD4+ and CD8+ T cells specific for the spike (S) protein of the SARS-CoV-2 in the peripheral blood of individuals with different antigen exposure histories. Antigen-specific cells were detected by peptide stimulation and subsequent detection of cytokine expression by multiparameter flow cytometry. Four major functions of the T cells were monitored; cytotoxicity (CD107a and IFNγ expression), IFNγ-expression, IL-2 expression, and TNFα expression (Fig. 4a). Detailed gating strategy for identification of antigen-specific T cells is provided as Supplemental Fig. 3. Interestingly, we did not observe any significant differences between the antigen exposure groups for the S-specific CD4+ T cells regardless of their function. Nevertheless, a similar trend was observed in the cases of cytotoxic, IFNγ-expressing and IL-2-expressing CD4+ T cells as for the S1-specific plasma IgG; 2xVacc+δ, 3xVacc, 3xVacc+o, and 3xVacc+δ were higher than the baseline 2xVacc group and the groups with an alpha breakthrough infection; 2xVacc+α, 3xVacc+α (Fig. 4b). Also, the frequencies of S-specific CD8+ T cells were not significantly different between the antigen exposure groups. However, there was a trend suggesting that the 3xVacc+o group had the highest median frequency of those cells for all groups (Fig. 4c). We have previously demonstrated that unvaccinated individuals infected with SARS-CoV-2 develop high frequencies of the nucleocapsid-specific T cells[31,32]. To check whether this is also true for breakthrough infections we stimulated the peripheral blood T cells with peptides spanning the entire sequence of the SARS-CoV-2 nucleocapsid (N) protein. Antigen-specific cells were detected by monitoring the same functions as for the S protein. Strikingly, we observed no significant differences between the antigen exposure groups with and without breakthrough infections indicating that previously vaccinated individuals either do not develop N-specific T cells or at very low levels. This was true for both CD4+ (Fig. 4d) and CD8+ (Fig. 4e) T cells. Regarding the overall SARS-CoV-2-specific T cell response (not discriminating the CD4+ and CD8+ T cell or their specificity for S or N proteins) 3xVacc group had significantly increased frequencies of cytotoxic (1.7-fold; $p = 0.01$) and IFNγ-expressing (2.1-fold; $p = 0.012$) cells compared to the 2xVacc group (Fig. 4f). The largest proportion of SARS-CoV-2-specific cytotoxic T cells (discriminated were CD4+ S-specific, CD8+ S-specific, CD8+ N-specific, and CD4+ N-specific T cells) represented CD4+ S-specific T cells for 2xVacc, 2xVacc+α, 2xVacc+δ, and 3xVacc groups ($p < 0.01$ for all comparisons). The same cell population prevailed among the IFNγ-expressing cells ($p < 0.05$ for all comparisons) and IL-2-expressing cells ($p < 0.0001$ for all comparisons) for all antigen exposure groups. Similarly, in the case of TNFα-expressing T cells, CD4+ cells specific for the S-protein represented the large majority of the response for all ($p < 0.05$ for all comparisons) except the 2xVacc+δ group. Comparing the antigen exposure groups

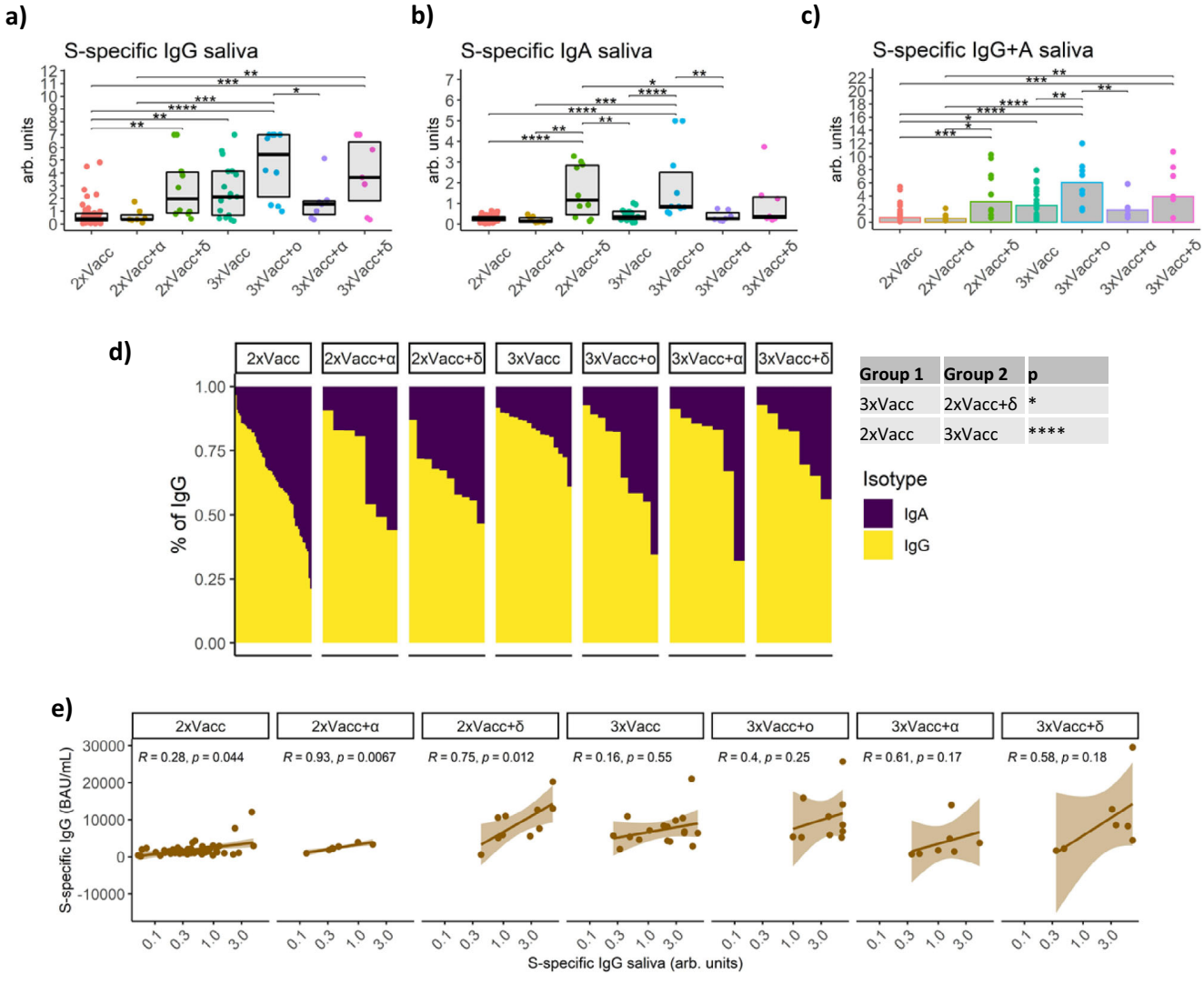


Fig. 2 | SARS-CoV-2-specific antibodies in saliva. S1-specific **a** IgG (exact *p* values in sequential order from the upmost bracket: $^{**}p = 0.0091$, $^{***}p = 0.0003$, $^{*}p = 0.015$, $^{***}p < 0.00012$, $^{****}p < 0.0001$, $^{**}p = 0.0032$, $^{**}p = 0.0091$), **b** IgA (exact *p* values in sequential order from the upmost bracket: $^{**}p = 0.0015$, $^{*}p = 0.019$, $^{****}p < 0.0001$, $^{***}p < 0.00027$, $^{****}p < 0.0001$, $^{**}p = 0.0027$, $^{**}p = 0.0044$, $^{****}p < 0.0001$), and **c** IgG +IgA (exact *p* values in sequential order from the upmost bracket: $^{**}p = 0.0098$, $^{***}p = 0.00041$, $^{**}p < 0.0015$, $^{**}p < 0.0023$, $^{****}p < 0.0001$, $^{****}p < 0.0001$, $^{*}p < 0.017$, $^{*}p < 0.016$, $^{***}p < 0.00034$) levels in saliva for the seven groups with different antigen exposure histories. Data is displayed as boxplots (**a**, **b**), indicating the first quartile, median, and third quartile, or bars showing the median (**c**) with individual data points. **d** Relative proportions of IgA and IgG isotypes among the S1-specific antibodies in the saliva of individuals with different antigen exposure histories. Data are shown as a stacked bar plot for each individual. Statistically significant differences between the groups are indicated in the table next to the graph ($^{*}p = 0.011$, $^{****}p < 0.0001$). **e** Correlations between the plasma and salivary S1-specific IgG for the seven antigen exposure groups. The 95% confidence intervals around the line of best fit are displayed as shading. The *r* and *p* values are given for each line. The following numbers of biologically independent samples were included in each group for all the graphs in this figure: 2xVacc, $n = 51$; 2xVacc+α, $n = 7$; 2xVacc+δ, $n = 10$; 3xVacc, $n = 17$; 3xVacc+o, $n = 10$; 3xVacc+α, $n = 7$; 3xVacc+δ, $n = 7$. Differences between the groups were assessed using the two-sided Mann–Whitney test with Holm's correction for multiple testing. The strength of correlations was assessed by the two-sided Spearman's correlation test. Source data are provided as a Source data file.

no significant differences in proportions of the four cell populations were observed for any of the functions (Fig. 4g). Most of the study participants had detectable S-specific CD4+ T cells for all of the measured functions (Fig. 4h). Particularly high proportions were observed within the 3xVacc, 3xVacc+o, and 3xVacc+δ groups. The second most frequent response was CD8+ T cells specific for the S-protein. Here, the 3xVacc+o, 3xVacc+α, and 3xVacc+δ groups harbored the highest percentage of responders. More rare were individuals with detectable N-specific CD4+ and CD8+ T cells, especially within the 2xVacc and 2xVacc+α groups (Fig. 4h).

To sum up, additional antigen exposures of twice-vaccinated individuals do not significantly boost the frequencies of SARS-CoV-2-specific T cells except in the case of three vaccine doses. CD4+ T cell responses were more frequent than CD8+ T cells and more T cells were specific for S than N protein. Individuals with omicron breakthroughs had an increased proportion of N-specific T cells.

Individuals with breakthrough infections have a better correlated adaptive immune response

The quality of antigen exposure is not only dependent on the magnitudes of individual immune components but also on their coordination. Apart from antibodies, many studies have demonstrated the importance of SARS-CoV-2-specific memory B and T cells[28–30]. Considering their distinct mechanism of action a multilayer immune response might be more effective at preventing SARS-CoV-2 infection.

We, therefore, correlated the measured immune parameters including antibody, memory B cell, and T cell responses for each of the seven groups with different antigen exposure histories. For the 2xVacc and 3xVacc groups, we observed a moderate to low degree of

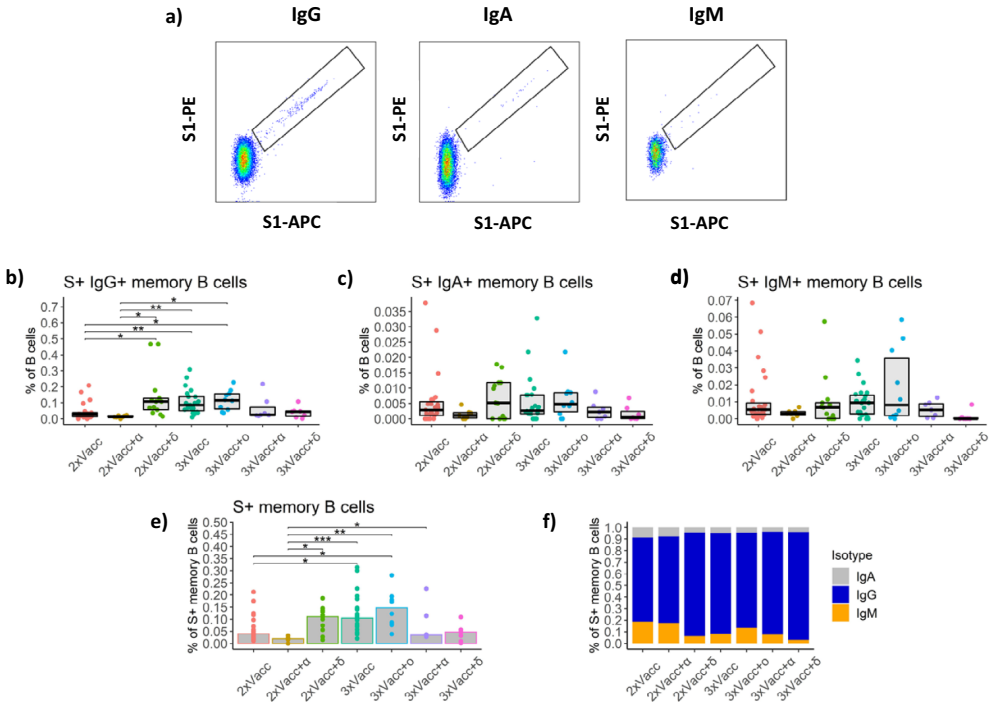


Fig. 3 | SARS-CoV-2-specific memory B cell response after different combinations of vaccination and breakthrough infection. a Representative flow cytometry pseudocolor plots for detection of S1-specific memory B cells with different BCRs. For the detailed gating strategy see Supplemental Fig. 2. Frequencies of **b** IgG+ (exact *p* values in sequential order from the upmost bracket: $^{*}p = 0.014$, $^{**}p = 0.005$, $^{*}p = 0.015$, $^{*}p = 0.014$, $^{**}p < 0.004$, $^{*}p = 0.047$), **c** IgA+, **d** IgM+, and **e** total S1-specific memory B cells (exact p values in sequential order from the upmost bracket: $^{*}p = 0.014$, $^{**}p = 0.005$, $^{*}p = 0.015$, $^{*}p = 0.014$, $^{**}p < 0.004$, $^{*}p = 0.047$) in peripheral blood of individuals belonging to the seven antigen exposure groups expressed as a percentage of B cells. The data is displayed as boxplots (**b**–**d**), indicating the first quartile, median, and third quartile, or bars showing the median (**e**) with individual data points. **f** Relative proportions of S1-specific memory B cells bearing BCRs of a different isotype (color-coded) presented as stacked bar plots. Each section of the bar represents the median proportion of an isotype. The following numbers of biologically independent samples were included in each group for all the graphs in this figure: 2xVacc, $n = 27$; 2xVacc +α, $n = 7$; 2xVacc+δ, $n = 13$; 3xVacc, $n = 23$; 3xVacc+o, $n = 10$; 3xVacc+α, $n = 7$; 3xVacc +δ, $n = 7$. Differences between the groups were assessed using the two-sided Mann–Whitney test with Holm's correction for multiple testing. Source data are provided as a Source data file.

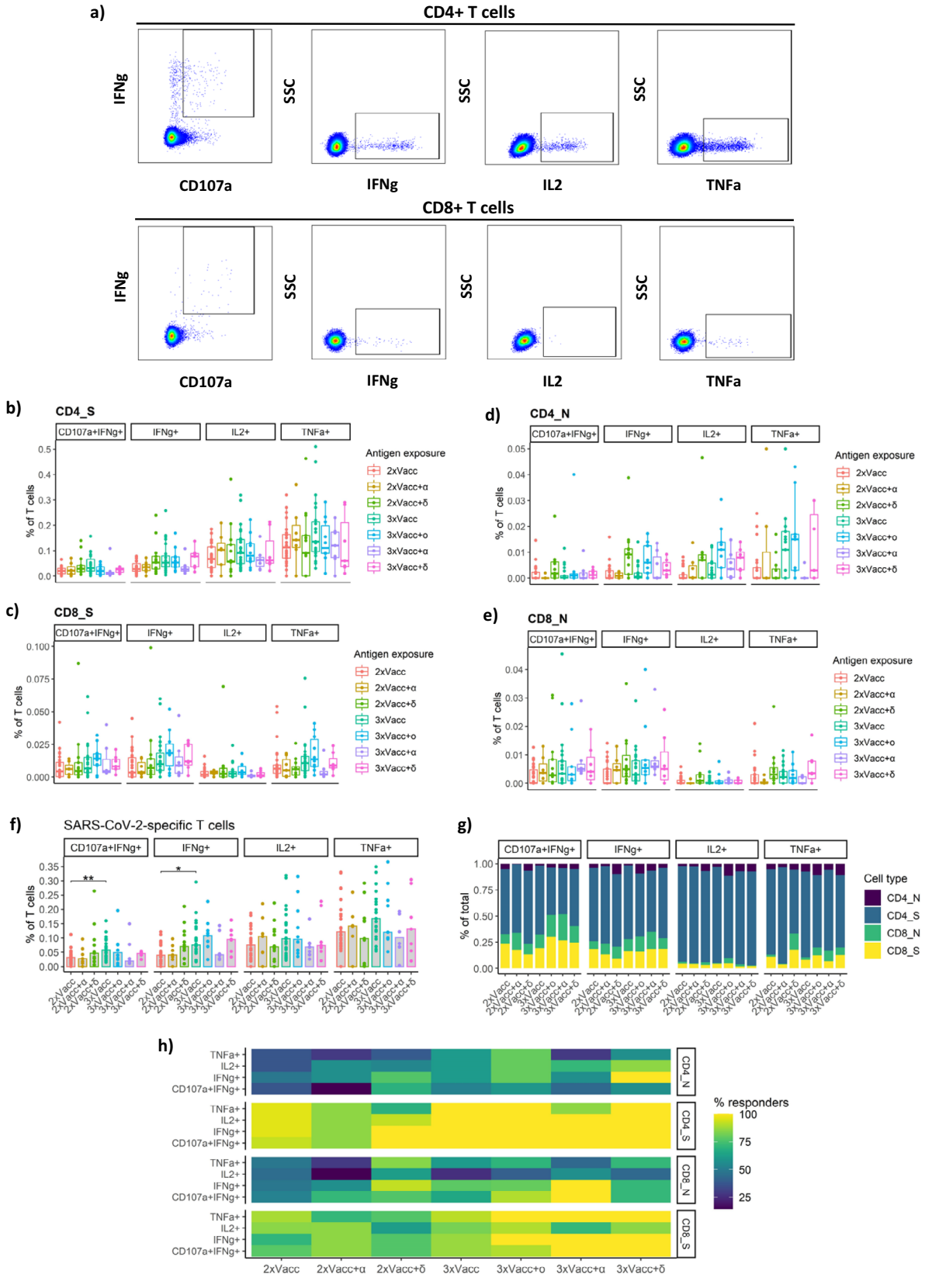
correlation among the parameters defining antibody response and among the parameters defining the T cell response. The rest of the immune response was poorly correlated (Fig. 5). For the rest of the antigen exposure groups, all with breakthrough infections, we observed a strong to moderate degree of correlation within the antibody compartment, and also the T cell compartment. For the 3xVacc +α and 3xVacc+δ groups, a considerable proportion of correlations among the T cell parameters was inverse (Fig. 5). Strong correlations between the antibodies and T cells and antibodies and memory B cells were mostly observed in the cases of the 2xVacc+δ, 3xVacc+δ, 2xVacc +α, and 3xVacc+α groups. Moreover, groups with breakthrough infections showed a higher degree of correlation between the memory B cell and T cell parameters compared to only vaccinated groups. Of note, 3xVacc+o, 3xVacc+δ, and 3xVacc+α groups had a high proportion of inverse correlations (Fig. 5). The parameters defining memory B cell response were weakly correlated regardless of antigen exposure group (Fig. 5).

Taken together, our data suggest a better correlated immune response among individuals with breakthrough infection when compared to those only vaccinated. Moreover, individuals with four antigen exposures had a higher proportion of inverse correlations.

Discussion

There is increasing evidence that multiple antigen exposures might be needed for robust immunity against SARS-CoV-2[16,18,20,23]. Furthermore, studies have suggested that hybrid immunity in terms of vaccination and infection offers improved protection from the disease[33,34]. It is, however, not clear how different combinations of vaccination and breakthrough infection with SARS-CoV-2 variants shape the immune response. Here we systematically compared the antibody, memory B cell, and T cell responses of individuals that initially received 2 doses of mRNA vaccine and were later boosted by either breakthrough infection, vaccination, or both. We discriminated the infections with alpha, delta, and omicron variants. Our findings suggest augmented immune responses among twice-vaccinated individuals that recovered from the delta variant infection and thrice-vaccinated individuals that recovered from omicron breakthrough infection.

The assessment of SARS-CoV-2 immunity most often relies on the measurement of spike-specific antibodies in plasma[24]. We have shown that, following the initial two doses of mRNA vaccine, a breakthrough infection with delta, or a third vaccine dose with or without additional omicron or delta breakthrough significantly boosts the production of spike-specific and neutralizing antibodies. Conversely, the alpha breakthrough infection did not significantly enhance antibody production. Furthermore, we have shown that the group with delta variant breakthrough infection most efficiently neutralized delta SARS-CoV-2, and the group with omicron breakthrough most efficiently neutralized the omicron virus. This suggests that the pre-existing immunity is shaped by the variant causing breakthrough infection. Further supporting this hypothesis, individuals who recovered from the infection with a particular variant showed a stronger correlation between the spike-specific antibody levels and neutralization against that variant. In line with previous studies, the currently circulating omicron variant was significantly more resistant to neutralization than wild-type or delta and delta was more resistant to neutralization than wild-type for vaccinated individuals only[35,36]. The only exception was the group with the omicron breakthrough that equally neutralized all three variants. Overall, these findings suggest superior humoral immunity against



SARS-CoV-2 for vaccinated individuals with delta or omicron breakthrough infections.

When evaluating protection from SARS-CoV-2 infection, the measurement of antibodies in saliva might be more informative since they can neutralize the virus immediately after it enters the upper respiratory tract[26,27]. We demonstrated increased levels of salivary anti-spike antibodies for the same antigen exposure groups as in the case of plasma IgG. Exceptionally high levels of spike-specific IgG and IgA in saliva were observed for the three times vaccinated individuals that recovered from omicron infection. This may be due to the increased replication of omicron in the upper respiratory tract compared to the previous variants and consequently stronger stimulation of the local mucosal immunity[37,38]. Moreover, given the abundance of IgG in comparison to IgA in the saliva our data demonstrate that the majority

Fig. 4 | SARS-CoV-2-specific T cell response to different antigen exposure scenarios. Frequencies of S-specific **a** representative flow cytometry pseudocolor plots demonstrating the detection of SARS-CoV-2-specific CD4+ and CD8+ T cells with different effector functions; cytotoxicity (CD107a and IFNγ expression), IFNγ-expression, IL-2 expression, and TNFα expression. For the detailed gating strategy see Supplemental Fig. 3. **b** CD4+ T cells and **c** CD8+ T cells with different effector functions as a percentage of bulk T cells. Frequencies of N-specific **d** CD4+ T cells and **e** CD8+ T cells with different effector functions as a percentage of bulk T cells. Different antigen exposure groups are color-coded. For panels **b**–**e**, the data is displayed as box-whisker-plots, indicating minimum, first quartile, median, third quartile, and maximum, with individual data points. **f** Frequencies of S or N-specific T cells with different functions, not discriminating the CD4+ and CD8+ T cells. The exact p values in sequential order from the leftmost bracket: $^{**}p = 0.01$, $^{*}p = 0.012$. Data is displayed as bars showing the median with individual data points. **g** Relative proportions of S-specific CD4+, N-specific CD4+, S-specific CD8+, and N-specific CD8+ T cells performing different functions for each antigen exposure group. Data are presented as stacked bar plots. CD4+/CD8+ T cells and their specificities for N or S proteins are color-coded. **h** Percentage of individuals with detectable SARS-CoV-2-specific CD4+ and CD8+ T cells within each group. The percentage of responders (individuals where the frequency of stimulation-responding T cells was higher than in the negative control) is color-coded. The following numbers of biologically independent samples were included in each group for all the graphs in this figure: 2xVacc, $n = 27$; 2xVacc+α, $n = 7$; 2xVacc+δ, $n = 13$; 3xVacc, $n = 23$; 3xVacc +o, $n = 10$; 3xVacc+α, $n = 7$; 3xVacc+δ, $n = 7$. Differences between the groups were assessed using the two-sided Mann–Whitney test with Holm's correction for multiple testing. Source data are provided as a Source data file.

of the spike binding activity in saliva was by IgG and not IgA antibodies regardless of the antigen exposure history. The highest proportion of IgA spike binding was among the twice-vaccinated individuals, while the third vaccination did not lead to a further increase in IgA immunity. Interestingly, we observed that salivary spike-specific IgG does not correlate well with the plasma IgG levels for most of the antigen exposure groups, suggesting that a significant part of the salivary IgG is locally produced.

Following the SARS-CoV-2 infection or vaccination, the antibody levels rapidly decline increasing the chance of breakthrough infections[10,11,31]. This is, however, not true for the memory B cells that are more persistent and therefore particularly important for long-term protection against severe SARS-CoV-2 infection[28,31]. We found that most of the spike-specific memory B cells found in the peripheral blood of vaccinated individuals with or without breakthrough infections bear IgG BCR. The highest frequencies of those cells were observed for the groups with strong antibody responses except for the thrice vaccinated group that recovered from delta infection. A similar trend was observed for the IgA-expressing spike-specific memory B cells but was not statistically significant due to the large intraindividual variability. Together with the antibody measurements, the memory B cell data implies that the fourth antigen exposure does not further augment the B cell immunity established after three antigen exposures, except in the case of the breakthrough with a genetically distinct omicron variant.

Besides antibodies, T cells represent an important mechanism for limiting viral infections and were previously associated with protection from SARS-CoV-2 infection[29,30]. Importantly, the frequency of these cells remains at elevated levels for a longer time after infection or vaccination than the antibody level[31,39]. Our data show that further antigen exposures, either by infection or vaccination, of the twice-vaccinated individuals, mostly do not improve the SARS-CoV-2-specific T-cell response. The only exception was the thrice vaccinated group that had significantly elevated cytotoxic and IFNγ-secreting SARS-CoV-2-specific T cells. Of note, SARS-CoV-2-specific CD4 + T cells were considerably more frequent than the CD8 + T cells and T cells specific for the spike protein prevailed over those recognizing the nucleocapsid protein. This is in contrast to the only infected individuals that develop equal levels of nucleocapsid- and spike-specific CD4 + T cells as observed in our previous studies[31,32]. Moreover, the frequency of these cells is considerably higher in only infected individuals suggesting that vaccination impairs the formation of nucleocapsid-specific T cells possibly due to the reduced viral replication and availability of the antigen. Interestingly, some of the vaccinated individuals without breakthrough infection had detectable nucleocapsid-specific T cells which could be explained by the high levels of pre-existing naïve or cross-reactive T cells previously documented among SARS-CoV-2 naïve individuals[30,40]. To sum up, individuals boosted by infection or vaccination do not have significantly augmented T cell immunity compared to the subjects that received only two vaccine doses.

The quality of immunity against viral infections does not only depend on a single but rather on the synchronization of multiple immune mechanisms. We demonstrated that individuals with breakthrough infections have better correlated parameters defining the adaptive immune response than those only vaccinated. Increased coordination of the immune response after breakthrough infection complies with the findings demonstrating better protection among individuals with hybrid immunity[33,34].

Taken together we compared the adaptive immune response of individuals with different antigen exposure histories in terms of vaccination and breakthrough infection. Our findings suggest that delta but not an alpha breakthrough infection or third vaccination of doubly vaccinated individuals considerably improves SARS-CoV-2 immunity. Strikingly, the fourth antigen exposure did not further augment the immune response compared to the three antigen exposures except for the omicron breakthrough infection. The observed differences between the groups with different antigen exposure scenarios are likely due to a combination of factors such as antigenic distance, the severity of breakthrough infection, the immunogenicity of the SARS-CoV-2 variants, and the time passed between the antigen exposures. No biases towards high-risk populations (elderly, immunosuppressed) were identified for any of the groups. Limitations of this study are that it is observational and does not reveal the mechanism driving improved immune response in particular groups. The study participants were employed at the University Hospital Bonn, therefore, the cohort was biased toward higher-educated individuals, aged between 19 and 69 years which likely does not reflect the general population. Moreover, we did not measure the neutralization capacity against the alpha variant, and some of the compared groups have relatively low sample numbers. Strengths of the study are that the participants were monitored for SARS-CoV-2 infections by RT-PCR and self-testing throughout the pandemic and that the SARS-CoV-2 variants were identified by sequencing rather than the time point of infection.

Methods

Study cohort

A total of 110 individuals that were initially immunized with 2 doses of mRNA-based SARS-CoV-2 vaccine and subsequently infected and/or vaccinated were recruited for the study. The recruitment was conducted by the occupational healthcare department of the University Hospital Bonn. The first contact was established by telephone after which a written invitation and a consent form were sent to each participant. All individuals were sampled 2–9 weeks following the last antigen exposure. The individuals belonging to different study arms were preselected so that the times from the last antigen exposure did not significantly differ between the groups. Age or sex was not among the selection criteria and no significant differences in age and sex distribution were observed between the groups. Detailed information on the antigen exposure and sampling time points as well as demographic information is provided in Supplemental Fig. 1 and Supplemental Table 1. Breakthrough infections were confirmed by RT-PCR

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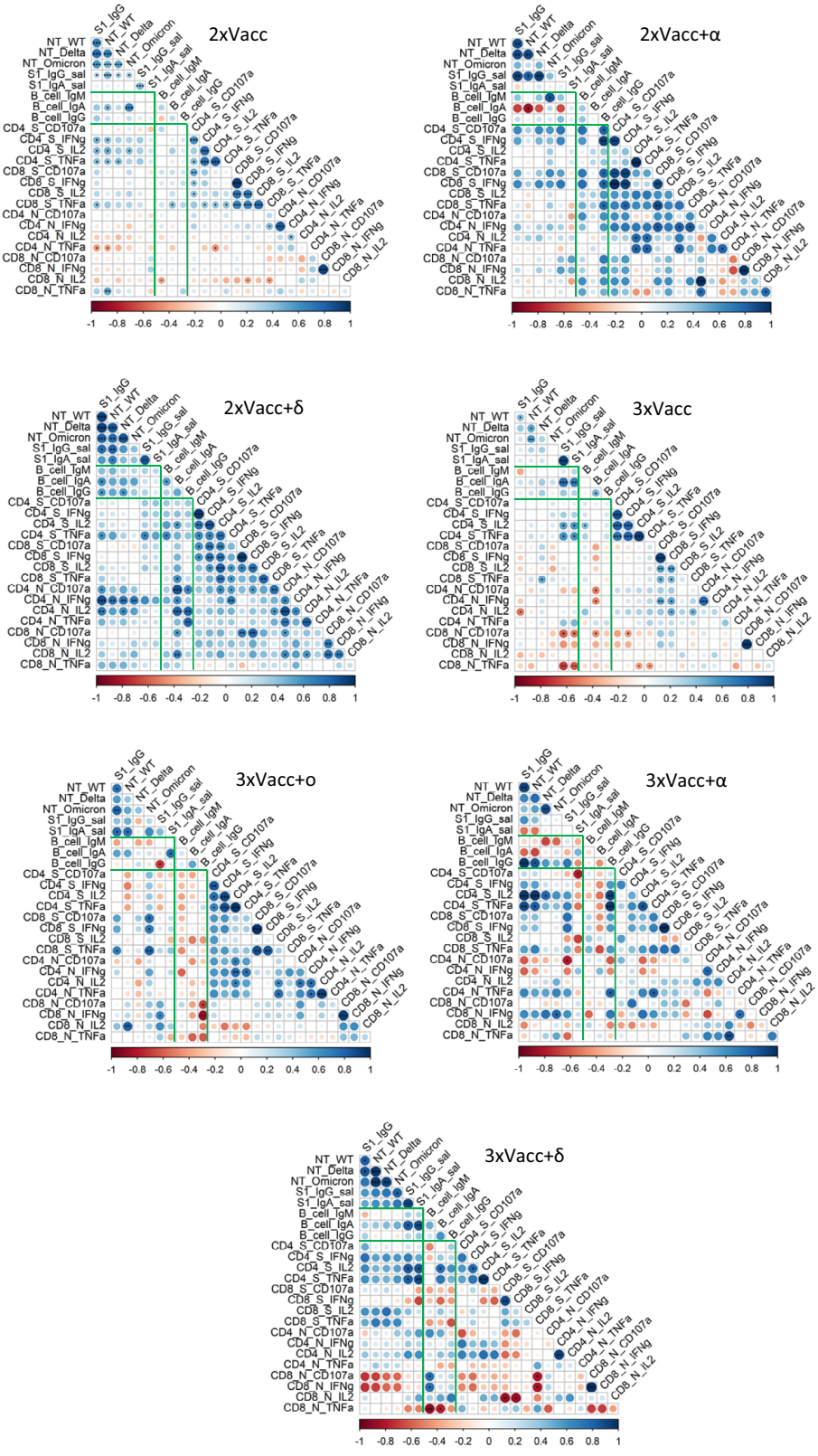

Fig. 5 | Correlation between the parameters of the adaptive immune response to different antigen exposure scenarios. Correlation matrices demonstrate the strength of correlations between the measured immune parameters for each of the antigen exposure groups. The strength of a correlation (Spearman's correlation coefficient) is depicted by the size and color of the circle, significance is indicated by asterisks. The exact *p* values are given in the Source data file. Green lines separate correlations between different branches of the measured immune parameters; antibodies, memory B cells, and T cells. The following numbers of biologically independent samples were included in each group for all correlated parameters: 2xVacc, $n = 54$ (for plasma antibodies), 2xVacc, $n = 51$ (for plasma antibodies), 2xVacc, $n = 27$ (for B and T cells); 2xVacc+α, $n = 7$; 2xVacc+δ, $n = 13$ (for plasma antibodies, B cells, and T cells), 2xVacc+δ, $n = 10$ (for saliva antibodies); 3xVacc, $n = 23$ (for plasma antibodies, B cells, and T cells), 3xVacc, $n = 17$ (for saliva antibodies); 3xVacc+o, $n = 10$; 3xVacc+α, $n = 7$; 3xVacc+δ, $n = 7$. The strength of correlations was assessed by the two-sided Spearman's correlation test. Source data are provided as a Source data file.

and the viral RNA was sequenced as a part of routine SARS-CoV-2 variant monitoring at the diagnostics department of the Institute of Virology, University Hospital Bonn. All participants were either employed or studied at the University Hospital Bonn at the time of sampling but were not necessarily healthcare workers. As employees of the University Hospital Bonn study participants were obliged to perform two antigen tests every week and RT-PCR whenever they developed symptoms similar to Covid-19. Furthermore, individuals with a history of previous SARS-CoV-2 infection were not taken into the study. All individuals that had a breakthrough infection were infected only once. For the groups without breakthrough infections, only individuals without confirmed SARS-CoV-2 infection, and negative nucleocapsid ELISA results were included.

Ethics approval

The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn (ethics approval numbers 125/21) and all participants provided written informed consent. No compensation was provided for the participants.

Sample collection and storage

Study participants provided peripheral blood specimens, saliva, and pharyngeal swabs. Blood was centrifuged and EDTA-plasma was stored until analysis (−80 °C). Before saliva collection participants were instructed not to eat or drink for at least 60 min. Afterward, participants would retain saliva for 1–2 min and expectorate it in a centrifuge tube. Saliva samples were centrifuged to remove solid particles and frozen at −20 °C. PBMC were isolated by density gradient centrifugation and cryopreserved in liquid nitrogen.

Determination of SARS-CoV-2 S- and N-specific antibodies in plasma

N-specific antibody levels were assessed using the Roche Cobas® SARS-CoV-2 assay following the manufacturer's protocol. For the determination of S1-specific IgG, an in-house quantitative ELISA was used. For that microtiter plates with high-binding capacity were coated with 100 µl of coating buffer (carbonate-bicarbonate buffer, pH = 9.6) containing 1 µg/ml of recombinant SARS-CoV-2 S1 protein (Biotinylated SARS-CoV-2 (COVID-19) S1 protein, Acrobiolabs, S1N-C82E8-200ug-AC). Coated plates were covered and incubated overnight at 4 °C. After washing with wash buffer (PBS with 0.05% (v/v) Tween®−20) plates were blocked (PBS containing 1% (w/v) BSA) to prevent unspecific binding. Cryopreserved EDTA plasma samples were thawed and diluted 1:3200 in the blocking buffer. Blocked plates were washed, incubated with plasma and standard samples, washed again, and incubated with 100 µl HRP-conjugated anti-IgG antibody (Goat anti-Human IgG (Heavy chain) Secondary Antibody, HRP, Invitrogen, A18805) diluted 1:8000 in wash buffer. Incubation steps were performed for 1 h at 37 °C. Afterward, plates were washed and 100 µl of the substrate solution was added (TMB Chromogen Solution, Life technologies, 002023). The reaction took place at room temperature for 5 min until the addition of 50 µl of 0.2 M H_2SO_4. Finally, optical density at 450 nm was measured. The background-subtracted OD_{450} readings were interpolated to the standard dilution curve calibrated to the international WHO standard (NIBSC reference number: 20/136). The positivity cutoff was determined based on measurements of plasma samples from healthy individuals collected before the Covid-19 outbreak. All samples were measured in duplicates.

Determination of SARS-CoV-2 S-specific IgG and IgA in saliva

The relative amounts of S1-specific IgA and IgG in saliva were measured by in-house quantitative ELISA. High-binding microtiter plates were coated with 100 µl of coating buffer (carbonate-bicarbonate buffer, pH = 9.6) containing 1 µg/ml of recombinant SARS-CoV-2 S1 protein (Biotinylated SARS-CoV-2 (COVID-19) S1 protein, Acrobiolabs, S1N-C82E8-200ug-AC). After overnight incubation at 4 °C the plates were washed (PBS with 0.05% (v/v) Tween®−20), blocked (PBS containing 3% (w/v) BSA), and washed again. Frozen saliva samples were thawed, diluted in sample buffer (PBS containing 1% (w/v) BSA), and pipetted onto the plate. Following incubation with saliva samples and standard dilutions plates were washed and incubated with 100 µl HRP-conjugated anti-IgG antibody (Goat anti-Human IgG (Heavy chain) Secondary Antibody, HRP, Invitrogen, A18805) diluted 1:8000 in wash buffer or 100 µl HRP-conjugated anti-IgA antibody (Goat anti-Human IgA (Heavy chain) Secondary Antibody, HRP, Invitrogen, A18781) diluted 1:1000 in wash buffer. Plates were then washed and 100 µl of the substrate was added (TMB ELISA Substrate, High Sensitivity, Abcam, ab171523). The reaction took place at room temperature for 5 min, followed by the addition of 100 µl of 1 M H_2SO_4. Finally, optical density at 450 nm was measured. The background-subtracted OD_{450} readings were interpolated to the standard dilution curve. The cutoff for positivity was determined based on measurements of saliva samples from healthy individuals collected before the Covid-19 pandemic. The same concentrations (15 ng/ml) of S1-specific monoclonal antibody (anti-SARS-CoV-2-RBD antibody, clone CR3022, Abcam, ab278112/ab273073) with IgA or IgG constant region was measured to make the OD_{450} readings comparable between the assays. All samples were measured in duplicates.

Plaque reduction neutralization assay

The neutralization capacity of plasma samples was determined by a plaque reduction neutralization assay. Therefore, plasma was heat-inactivated for 30 min at 56 °C and serially two-fold diluted in OptiPRO SFM (Gibco, 12309-019) cell culture medium. A total of 10 dilutions between 2-fold and 32768-fold were measured for each sample depending on the neutralization capacity of a specimen. No further technical replicates were performed. Each dilution was combined with 80 plaque-forming units of SARS-CoV-2 (either wild-type, delta, or omicron variant) in OptiPRO SFM (Gibco, 12309-019) cell culture medium, incubated for 1 h at 37 °C, and added to Vero E6 cells (ATCC, CRL-1586). The cells were seeded in 24-well plates at 1.25×10^5 cells/well 24 h earlier. Following 1 h incubation at 37 °C, the inoculum was removed and cells were overlaid with a 1:1 mixture of 1.5% (w/v) carboxymethylcellulose in 2xMEM supplemented with 4% FBS. After incubation at 37 °C for three days, the overlay was removed and the 24-well plates were fixed using a 6% formaldehyde solution and stained with 1% crystal violet in 20% ethanol revealing the formation of plaques. The number of plaques was plotted against the serum/supernatant dilutions and IC50 was determined using GraphPad Prism software version 9.4.1 (681).

B cell isolation

B cells were enriched from cryopreserved PBMC samples by immunomagnetic isolation (REAlease® CD19 MicroBead Kit, human, Miltenyi Biotec, 130-117-034). Isolation was performed following the manufacturer's instructions. Briefly, PBMCs, which had been thawed and rested overnight, were resuspended in recommended isolation buffer and labeled with anti-CD19 antibodies coupled to magnetic beads. Labeled cells were then immobilized onto a magnetic column. B cell-depleted flow-through was used for the assessment of CD4 + T cell responses. Immobilized B cells were washed out of the column and enzymatically released from magnetic beads.

Detection of S1-specific memory B cells by flow cytometry

SARS-CoV-2 S1-specific B cells were identified by immunofluorescent tagging with recombinant wild-type SARS-CoV-2 S1 protein. Therefore, the magnetically isolated B cells were resuspended in FACS buffer (PBS supplemented with 2% FCS, 0.05% NaN_3, and 2 mM EDTA) and incubated with the fluorescently labeled recombinant SARS-CoV-2 S1 protein (Biotinylated SARS-CoV-2 (COVID-19) S1 protein, Acrobiolabs, S1N-

C82E8-200ug-AC). To minimize the unspecific binding of the probe, S1 protein was conjugated to two different streptavidin-fluorochrome conjugates, streptavidin-PE (Biolegend, 405204) and streptavidin-APC (Biolegend, 405207), in an equimolar ratio. After 15 min at 4 °C anti-IgG-BV421 antibody (clone G18-145, Biolegend, 562581, diluted 1:20) was added to the cell suspension and the incubation was continued for another 15 min. Following the binding of S1 probes, cells were washed with PBS and stained for viability (ZombieAqua, Biolegend, 423102) for 15 min at 4 °C. Subsequently, cells were washed with FACS buffer and incubated with a solution of antibodies blocking human Fc receptors (FcR block, Miltenyi Biotec, 130-059-901, diluted 1:10). After 10 min a mixture of fluorescently labeled antibodies binding to surface antigens of B cells was added. The mixture included the following fluorescently-labeled antibodies: anti-CD3-BV510 (clone UCHT1, Biolegend, 300448, diluted 1:40), anti-CD27-BV605 (clone O323, Biolegend, 302830, diluted 1:20), anti-IgM-BV785 (clone MHM-88, Biolegend, 314544, diluted 1:20), anti-IgA-VioBright 515 (clone REA1014, Miltenyi Biotec, 130-116-886, diluted 1:40), anti-CD21-PE-Cy7 (clone Bu32, Biolegend, 354912, diluted 1:160), and anti-CD19-APC-Cy7 (clone HIB19, Biolegend, 302218, diluted 1:80). The staining was performed at 4 °C for 15 min. Following incubation, the cells were washed again and acquired on a BD FACS Celesta flow cytometer with BD FACSDiva™ Software Version 8.0 (BD Bioscience). Possible longitudinal fluctuations in laser intensity were monitored every day before the experiment and were compensated using fluorescent beads (Rainbow beads, Biolegend, 422905). The data were analyzed with the FlowJo Software version 10.0.7 (TreeStar). The frequency of S-specific memory B cells was calculated by subtracting the average frequency of S-binding memory B cells in eight healthy donor samples collected before the outbreak of the SARS-CoV-2 pandemic. No technical replicates were performed due to the scarcity of the samples.

Ex vivo stimulation of T cells

Overnight-rested B-cell-depleted PBMC were seeded in 96-well U bottom plates and stimulated with wild-type SARS-CoV-2 PepTivator (Miltenyi Biotec, 130-127-951/130-126-698) overlapping peptide pools spanning the entire sequences of SARS-CoV-2 S or N proteins, in presence of anti-CD107a-APC (clone H4A3; Biolegend, 328620, diluted 1:40) antibody. One million cells were stimulated per condition and the final concentration of each peptide was 1 μg/ml for both peptide pools. Co-stimulatory antibodies (BD FastImmune™ CD28/CD49d, BD Bioscience, 347690) were added to a final concentration of 1 μg/ml. Stimulation was performed at 37 °C for 6 h. For each sample, an equally treated DMSO-stimulated negative control was included. As a positive control, cells were stimulated with PMA (20 ng/ml) (Sigma-Aldrich, P1585-1MG) and ionomycin (1 μg/ml) (Sigma-Aldrich, I3909-1ML). One hour into stimulation Golgi Stop (BD Bioscience, 554724) and Golgi Plug (BD Bioscience, 555029) were added (final concentration 1 μg/ml) to inhibit vesicular transport and prevent the secretion of the cytokines.

Detection of SARS-CoV-2-specific T cells by flow cytometry

Following stimulation, cells were washed with PBS and stained with Zombie Aqua (Biolegend) dye to discriminate viable cells. The staining was performed for 15 min at 4 °C. Subsequently, samples were washed with FACS buffer, fixed, and permeabilized in CytoFix/CytoPerm Solution (BD Bioscience, 554714) for 15 min at 4 °C. Fixed cells were then washed with 1x Perm/Wash Buffer (BD Bioscience, 554723), and stained for intracellular markers for 15 min at 4 °C using the following antibody conjugates; anti-CD3-APC-Cy7 (clone UCHT1, Biolegend, 300426, diluted 1:40), anti-CD4-BV786 (clone SK3, BD Bioscience, 344642, diluted 1:40), anti-IFNγ-PE (clone B27, Biolegend, 506507, diluted 1:40), anti-TNFα-BV421 (clone Mab11, Biolegend, 502932, diluted 1:80), and anti-IL2-AF488 (clone MQ1-17H12, Biolegend, 500304, diluted 1:20). Each antibody was checked for performance and titrated before use. Finally, cells were washed with PBS and acquired on a BD FACS Celesta with BD FACSDiva™ Software Version 8.0 (BD Bioscience). Frequencies of antigen-specific CD4 + T cells were calculated as negative-control-subtracted data. Possible longitudinal fluctuations in laser intensity were monitored daily before the experiment using fluorescent beads (Rainbow beads, Biolegend, 422905). If needed PMT voltages were adjusted to ensure constant signal intensity over time. The data were analyzed with the FlowJo Software version 10.0.7 (TreeStar). No technical replicates were performed due to the scarcity of the samples.

Statistical analysis

Statistical analysis was performed using RStudio 2021.09.0 Build 351 software[41]. Differences between the groups were assessed using the Mann–Whitney test or Wilcoxon test for matched data with Holm's correction for multiple testing. All tests were performed two-sided. The strength of correlations was evaluated by Spearman's test. Statistical significance is indicated by the following annotations: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data contain information that could compromise the privacy of research participants. Data sharing restrictions imposed by national and transnational data protection laws prohibit the general sharing of data. However, upon submission of a proposal to the corresponding author and approval of this proposal by (i) the principal investigator, (ii) the Ethics Committee of the University of Bonn, and (iii) the data protection officer of the University Hospital Bonn, data collected for the study can be made available to other researchers. A source data file containing the statistics presented in the figures and a Supplemental table containing demographic information are provided with this paper. Source data are provided with this paper.

Code availability

No custom code or mathematical algorithm was generated for this study.

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Author contributions

Conceptualization: J.P., W.M.P., and H.S.; methodology: J.P., G.A., and H.S.; investigation: J.P., W.M.P., K.P., J.Z., M.B., C.B.S., and H.P.; resources: J.P. and W.M.P.; writing—original draft: J.P.; writing—review & editing: J.P., H.S., and G.A.; funding acquisition: J.P. and H.S.; supervision: H.S.

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Competing interests

The authors declare no competing interests. The idea, the plan, the concept, the protocol, the conduct, the data analysis, and the writing of the manuscript of this study were independent of any third parties, including the funding agency.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-023-36250-4.

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3. Danksagung

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Den unermüdlichen Mitarbeiterinnen und Mitarbeitern des Universitätsklinikums Bonn danke ich sehr für die Teilnahme an der Studie und deren Einsatz während der COVID-19-Pandemie. Den Kolleginnen und Kollegen des Betriebsärztlichen Dienstes sowie der „Corona-Abstrich-Ambulanz“ des Universitätsklinikums Bonn gilt zudem mein herzlicher Dank für die Unterstützung und die gute Zusammenarbeit während der Pandemie.

Abschließend möchte ich vor allem meiner Familie danken, die mich immer unterstützt hat.

4. Publikationsliste

1. Monzón-Posadas WO, Zorn J, Peters K, Baum M, Proksch H, Schlüter CB, Menting T, Pušnik J, Streeck H. Longitudinal monitoring of mRNA-vaccine-induced immunity against SARS-CoV-2. Front Immunol. 2023 Jan 19; 14:1066123. DOI: 10.3389/fimmu.2023.1066123
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5. Anhang

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Anamnesebogen bei Verdacht auf SARS-CoV-2 Infektion nach erfolgter Grundimmunisierung
(alle Angaben sind Pflichtfelder)

Name, Vorname: Geburtsdatum:

Datum der ersten Impfung: _____ Impfstoff: SARS-CoV-2-Impstoff (mRNA-1273)

Datum der zweiten Impfung: _____ Impfstoff: SARS-CoV-2-Impstoff (mRNA-1273)

Datum der 1. Auffrischimpfung: _____ Impfstoff: _____

Risiko-Kontakte in den letzten 48 h

Bestand **Kontakt** zu einer **positiv getesteten symptomatischen** Person?	ja mit PSA	ja ohne PSA	nein
Handelt es sich bei der Kontaktperson um eine/n	UKB-Mitarbeiterin/er?	Patientin/en?	Privaten Kontakt?
Rückkehr aus einem **Risikogebiet**? Wenn ja, **Land/Region**?		ja	nein
Wann war der **Kontakt**?			

Klinische Symptomatik

Haben Sie aktuell **Beschwerden**?	ja	nein
Leiden Sie aktuell an **Husten**, **Schnupfen**, **Halsschmerzen**, **Gliederschmerzen**, **Kopfschmerzen**?	ja	nein
Seit **wann**?		
Haben Sie aktuell **Temperatur**?	ja	nein
Seit **wann**?	bis 38,5°C	über 38,5°C
Besteht aktuell Geschmacksverlust / Geruchsverlust?	ja	nein
Weitere Symptome: Appetitlosigkeit, Gewichtsverlust, Übelkeit, Bauchschmerzen, Erbrechen, Durchfall, Konjunktivitis, Hautausschlag, Lymphknotenschwellung, andere, wenn ja welche?	ja	nein

Datum, Ort Unterschrift

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Personalfragebogen für Probandinnen und Probanden

(alle Angaben sind Pflichtfelder)

Name, Vorname: Geburtsdatum:

Statusgruppe:

☐ UKB-Beschäftigte ☐ DRK ☐ PJ-Studierende ☐ Sonstige Studierende ☐ Sonstiger Arbeitgeber und zwar: _____

Berufsgruppe:
MFA/ZFA/MTRA ☐ Arzt/Ärztin ☐ Pflege ☐

☐ MTLA/PTA/BTA etc. ☐ Patiententransport ☐ Reinigungs-/Wirtschaftsdienst, Haustechnik, Küche

☐ Verwaltung ☐ Sonstige: _____

Klinik, Institut oder Abteilung:

Teilnehmer/in-Nummer:

Bitte erstellen Sie hier Ihren eigenen Pseudoanonymisierungscode.

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Datum: 21.12.2021
KRa/Ro

Aktenzeichen: 125/21 (bitte stets angeben!)
Betr.: Ihr Antrag an die Ethik-Kommission
Studientitel: Die Wirksamkeit des Impfstoffs mRNA-1273 (Moderna) bei Probandinnen und Probanden des Universitätsklinikums Bonn (UKB) in Bezug auf die Inzidenz von SARS-CoV-2- Infektionen ~~mit den Virusvarianten B.1.1.7 und B.1.351 nach abgeschlossener Grundimmunisierung~~ mit den besorgniserregenden Virusvarianten (variants of concern, VOC) nach abgeschlossener Grundimmunisierung

Auflistung der eingereichten Unterlagen siehe Anhang

Hier: Ihr Schreiben vom 20.12.2021
- Studienerweiterung mit Anpassung des Studientitels

Sehr geehrte Frau Professorin Menting,

die Ethikkommission für klinische Versuche am Menschen und epidemiologische Forschung mit personenbezogenen Daten der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn hat die o. g. nachträgliche Änderung geprüft.

Es bestehen keine berufsrechtlichen oder berufsethischen Bedenken gegen die weitere Durchführung dieser Studie einschließlich der oben genannten nachträglichen Änderung.

Im Übrigen verweisen wir auf unser Votum vom 01.04.2021.

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Montag-Donnerstag
9:00-12:00 Uhr

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Mit freundlichen Grüßen

Prof. Dr. Kurt Racké
Vorsitzender der Ethikkommission

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9:00 – 12:00 Uhr

Datum:21. April 2021
KRa/Ro

Aktenzeichen: 125/21 (bitte stets angeben!)
Betr.: **Ihr Antrag an die Ethik-Kommission**
Studientitel: **Die Wirksamkeit des Impfstoffs mRNA-1273 (Moderna) bei Probandinnen und Probanden des Universitätsklinikums Bonn (UKB) in Bezug auf die Inzidenz von SARS-CoV-2- Infektionen mit den Virusvarianten B.1.1.7 und B.1.351 nach abgeschlossener Grundimmunisierung**

Hier: Ihre Email vom 20.04.2021
Auflistung der eingereichten Unterlagen siehe Anlage

Sehr geehrte Frau Professorin Menting,

die Ethik-Kommission für klinische Versuche am Menschen und epidemiologische Forschung mit personenbezogenen Daten der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn hat die n. g. Unterlagen geprüft und sieht die Punkte unseres Schreibens vom 01.04.2021 ausreichend beantwortet.

Im Übrigen verweisen wir auf unser Votum vom 01.04.2021

Mit freundlichen Grüßen

K. Racké

Prof. Dr. K. Racké
Vorsitzender der Ethik-Kommission

UNIVERSITÄT BONN

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Auflistung der eingereichten Unterlagen

Eingang am 23.03.2021

Anschreiben vom 22.03.2020
Antrag einschließlich Informationsschrift und Einwilligungserklärung

Eingang 20.04.2021

Email vom 20.04.2021
Probandeninformation und Einwilligungserklärung vom 15.04.2021

Rheinische Friedrich-Wilhelms-Universität
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E t h i k – K o m m i s s i o n

53127 Bonn, den 01.04.2021

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KRa/Ro

Lfd. Nr. 125/21
Bitte stets angeben!

Betr.: **Ihr Antrag an die Ethik-Kommission**
Studientitel: **Die Wirksamkeit des Impfstoffs mRNA-1273 (Moderna) bei Probandinnen und Probanden des Universitätsklinikums Bonn (UKB) in Bezug auf die Inzidenz von SARS-CoV-2- Infektionen mit den Virusvarianten B.1.1.7 und B.1.351 nach abgeschlossener Grundimmunisierung**

Hier: Ihr Schreiben vom 22.03.2021
Auflistung der eingereichten Unterlagen siehe Anlage

Sehr geehrte Frau Dr. Tanja Menting,

Die Ethik-Kommission für klinische Versuche am Menschen und epidemiologische Forschung mit personenbezogenen Daten der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn ist nach Beratung des o.g. Antrags auf ihrer Sitzung am 31.03.2021 zu dem Beschluss gekommen, gegen die o.g. Studie keine berufsethischen oder berufsrechtlichen Bedenken zu erheben, wenn die nachfolgenden Punkte berücksichtigt werden.

Personalfragebogen für Probandinnen und Probanden

1) Datendokumente, die als „Studiendokumente" nicht in der betriebsärztlichen Akte der Mitarbeiter verbleiben, müssen entsprechend der datenschutzrechtlichen Vorgaben (Erwägungsgrund 156 und Artikel 89 Nr. 1 DSGVO; § 6 Abs. 4 GDSG NRW) zumindest pseudonymisiet werden.

Informationsschrift und Einwilligungserklärung

2) Die Informationsschrift und Einwilligungserklärung sollte ein eigenständiges Dokument mit fortlaufender Seitennummerierung sein und eine Versionsdatierung tragen.
3) Es ist unpassend suggestiv, den potentiellen Studienteilnehmer zu Beginn der Informationsschrift bereits mitzuteilen, dass man sich über die Studienteilnahme freue, da eine informierte Entscheidung zur Teilnahme erst nach Information erfolgen kann.
4) Die Ausführungen zum Versicherungsschutz sind so nicht zutreffend. Es sollte darauf hingewiesen werden, dass Schädigungen im Zusammenhang mit fremdnützigen, studienbedingten Blutentnahmen gemäß § 2 Abs. 1 nr. 13b SGB VII unter dem Schutz der gesetzlichen Unfallversicherung stehen können, der auch etwaige Wegeunfälle einschließt, die auf direktem Weg vom und zum Ort der Probenentnahme auftreten und dass mögliche Forderungen an die Unfallkasse NRW, Postfach 33 04 20, 40437 Düsseldorf zu stellen sind.
5) Auf mögliche Konsequenzen, die sich auf Grundlage des IFSG bei einem positiven Erregerbefund ergeben, sollte hingewiesen werden.
6) Die datenschutzrelevanten Passagen der Einwilligungserklärung müssten gemäß Artikel 7 Nr. 2 DSGVO drucktechnisch hervorgehoben werden.
7) Die Einwilligungserklärung sollte vom dem/der aufklärenden Arzt*in gegengezeichnet werden, deren Namen auch leserlich angegeben sein sollte.

Datenschutzrechtliche Aspekte von Forschungsvorhaben werden durch die Ethikkommission grundsätzlich nur kursorisch geprüft. Voten / Bewertungen durch die Ethik-Kommission ersetzen mithin nicht die Konsultation des zuständigen Datenschutzbeauftragten.

Änderungen im Prüfplan müssen der Ethik-Kommission mitgeteilt werden und bedürfen der erneuten Beratung.
Des Weiteren müssen Änderungen bei den beteiligten Prüfärzten der Ethik-Kommission unverzüglich mitgeteilt werden.

Die ärztliche und juristische Verantwortung des Leiters der klinischen Prüfung und der an der Prüfung teilnehmenden Ärzte bleibt entsprechend der Beratungsfunktion der Ethik-Kommission durch unsere Stellungnahme unberührt.

Die Ethik-Kommission der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn arbeitet gemäß den nationalen gesetzlichen Bestimmungen und den ICH-GCP Richtlinien. Den Beratungen der Ethik-Kommission der Medizinischen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn liegt gemäß der gültigen Berufsordnung die maßgebende Deklaration des Weltärztebundes von Helsinki in der letzten revidierten Fassung zugrunde.

Mit freundlichen Grüßen

K. Racké

Prof. Dr. K. Racké
Vorsitzender der Ethik-Kommission

Nachfolgend sind die Mitglieder der Ethik-Kommission aufgeführt, die den o. g. Antrag auf ihrer Sitzung am 31.03.2021 beraten haben:

Herr Dr. M. Rademacher, Arzt für Neurologie
Frau Dr. A. Spottke, Ärztin für Psychiatrie und Psychotherapie
Frau Dr. Sarah. Thiele, Ärztin für Augenheilkunde
Frau Prof. Dr. Gadebusch-Bondio, Medizinethikerin
Herr Prof. Dr. H.-U. Spranger, Jurist
Herr F. Chmielewski, Apotheker
Herr Andreas Kocks, Patientenvertreter
Herr Prof. Dr. K. Racké, Arzt f. Pharmakologie u. Toxik. / Vors. der Ethik-Kommission

Auflistung der eingereichten Unterlagen

Eingang am 23.03.2021

Anschreiben vom 22.03.2020
Antrag einschließlich Informationsschrift und Einwilligungserklärung