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Durable immune responses after BNT162b2 vaccination in home-dwelling old adults

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ABSTRACT

Objectives: Elderly are an understudied, high-risk group vulnerable to severe COVID-19. We comprehensively analyzed the durability of humoral and cellular immune responses after BNT162b2 vaccination and SARS-CoV-2 infection in elderly and younger adults.
Methods: Home-dwelling old (n = 100, median 86 years) and younger adults (n = 449, median 38 years) were vaccinated with two doses of BNT162b2 vaccine at 3-week intervals and followed for 9-months. Vaccine-induced responses were compared to home-isolated COVID-19 patients (n = 183, median 47 years). Our analysis included neutralizing antibodies, spike-specific IgG, memory B-cells, IFN-γ and IL-2 secreting T-cells and sequencing of the T-cell receptor (TCR) repertoire.
Results: Spike-specific breadth and depth of the $CD4^+$ and $CD8^+$ TCR repertoires were significantly lower in the elderly after one and two vaccinations. Both vaccinations boosted IFN-γ and IL-2 secreting spike-specific T-cells responses, with 96 % of the elderly and 100 % of the younger adults responding after the second dose, although responses were not maintained at 9-months. In contrast, T-cell responses persisted up to 12-months in infected patients. Spike-specific memory B-cells were induced after the first dose in 87 % of the younger adults compared to 38 % of the elderly, which increased to 83 % after the second dose. Memory B-cells were maintained at 9-months post-vaccination in both vaccination groups. Neutralizing antibody titers were estimated to last for 1-year in younger adults but only 6-months in the older vaccinees. Interestingly, infected older patients (n = 15, median 75 years) had more durable neutralizing titers estimated to last 14-months, 8-months longer than the older vaccinees.
Conclusions: Vaccine-induced spike-specific IgG and neutralizing antibodies were consistently lower in the older than younger vaccinees. Overall, our data provide valuable insights into the kinetics of the humoral and cellular immune response in the elderly after SARS-CoV-2 vaccination or infection, highlighting the need for two doses, which can guide future vaccine design.
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Introduction

The rapid development and licensing of mRNA vaccines has resulted in a significant reduction in morbidity and mortality due

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to coronavirus disease 2019 (COVID-19) [1,2]. Based on preclinical studies of severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) [3], the spike protein was quickly identified as an antigenic target for SARS-CoV-2 vaccines. The elderly are at the highest risk of severe disease and death after COVID-19 [4,5], thus optimal protection for this group is a major goal of vaccine design and public health efforts. Despite this, the elderly were not included in the original COVID-19 vaccine licensure trials [6]. BNT162b2 vaccine induced a strong humoral response against SARS-CoV-2 in people over 80, while cellular responses were only detectable in 63 % [7]. Furthermore, humoral and cellular immune responses in older persons are lower compared to younger adults up to 6-months post-vaccination [8].

A reliable correlate of protection recognized by regulators has yet to be defined, however, there is compelling evidence in support of both humoral and cellular immunity in preventing severe disease [9,10]. Although protection against severe disease for the first few months after vaccination is well documented, the duration of vaccine-induced protection has been found to wane 6-months after vaccination [11]. It is currently unclear how the durability of humoral and cellular responses differs between younger adults and elderly persons after vaccination, and how this compares to immunity after infection.

We conducted a longitudinal study comparing the magnitude and durability of the immune responses elicited after vaccination and infection in different age groups. Here, we measured the immune response for up to 9-months after BNT162b2 vaccination in SARS-CoV-2 naive old and younger adults. We performed a comprehensive analysis of both humoral and cellular immune responses covering SARS-CoV-2 spike-binding and neutralizing antibodies, memory B-cells, functional T-cell responses and sequencing of the T-cell receptor (TCR) repertoire. Our data provide valuable information on the kinetics and durability of SARS-CoV-2 immune responses for younger adults and an understudied, high-risk, elderly group. We also compared the durability of vaccine-induced immune responses with those after mild-to-moderate infection in unvaccinated individuals, demonstrating that age differentially shapes immune responses after vaccination and infection.

Methods

Participants

We conducted a prospective cohort study of adults receiving pandemic COVID-19 vaccine (BNT162b2 Pfizer-BioNTech) between January and November 2021 from Eidsvåg general practice and Haukeland University Hospital in Bergen, Norway. All subjects provided written informed consent before inclusion in the study, which was approved by the regional ethics committee (Regional Committee for Medical Research Ethics, Northern Norway (REK Nord). The study is registered in the National Institute for Health database Clinical trials.gov (NCT04706390). The inclusion criteria were willingness to attend scheduled blood sampling visits and no previous SARS-CoV-2 infection. The exclusion criteria were history of anaphylaxis or hypersensitivity to vaccines. The infected cohort was recruited during March and April 2020 from home-isolated SARS-CoV-2 infected individuals and were followed clinically and immunologically at 2-, 6- and 12-months post-infection. The inclusion criteria were positive PCR test or antibody positivity at convalescence. All patients had mild-to-moderate infection and were not hospitalized, details are described elsewhere [12].

Vaccine

Each dose (0.45 ml) of BNT162b2 mRNA vaccine embedded in lipid nanoparticles contained 30 μg of a purified single-stranded, 5′-capped mRNA, encoding the spike protein from the first isolated SARS-CoV-2 strain (Genebank; Wuhan-Hu-1 strain, NC_045512).

Vaccine study design

All participants were vaccinated intramuscularly into the deltoid muscle with two doses of BNT162b2 mRNA vaccine at 3-week intervals. Subjects provided blood samples prior to and 3- and 6-weeks, 5- and 9-months after vaccination. Blood samples were collected using plastic serum tubes (BD Biosciences) and ethylene diamine tetra acetic acid (EDTA) tubes (BD Biosciences). A subgroup of the vaccination and infection cohorts provided a cell preparation tube (CPT) (BD Biosciences) for peripheral blood mononuclear cells (PBMCs) separation pre- and post-vaccination or post-infection, respectively, to examine MBC and T-cell responses. The infected cohort provided serum at 8-weeks, 6- and 12-months post-infection.

Clinical information

Electronic case report forms (eCRF) were developed using the Research Electronic Data Capture database (REDCap®) (Vanderbilt University, Nashville, Tennessee). The eCRF contained demographics, comorbidities, medication, exposure and infection history (RT-PCR results and presence of symptoms) and vaccination data.

Serological assays

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used for detecting spike-specific IgG [12,13]. Baseline sera were screened by Receptor Binding Domain (RBD) ELISA to test for seropositivity, positive samples were run in spike IgG ELISA. Endpoint titers were calculated as the reciprocal of the serum dilution giving an optical density value of 3 standard deviations above the mean of historical pre-pandemic sera (n = 128).

Microneutralization assay

The microneutralization (MN) assay was performed with an early isolated D614G strain (March 2020, Genebank; hCoV-19/Norway/Bergen-01/2020, lineage B.1, OM616023)(GISAID accession ID EPI_ISL_541970) in a certified Biosafety Level 3 Laboratory, as previously described [13]. Neutralizing titers were calculated as the reciprocal of the serum dilution giving 50 % inhibition of virus infectivity. For calculation purposes negative titers (<20) were assigned a value of 10.

Memory B-cell Enzyme-linked immunosorbent spot assay (ELISPOT)

PBMCs were resuspended in RPMI-1640 (Lonza) with 10 % fetal bovine serum (Hyclone), 100 U/ml penicillin, 0.1 mg/ml streptomycin (Sigma Aldrich) (negative control) or in medium containing 1 μg/ml R848 (MabTech) and 1 μg/ml rhIL-2 (MabTech) for expansion of B-cells. Two million cells were added per well in 24-well plates (Nunc) and incubated for 6 days at 37 °C, 5 % CO_2. ELISPOT plates (Millipore) were coated with 15 μg/ml anti-human IgG (MabTech), 10 μg/ml SARS-CoV-2 spike protein, or PBS only (negative control) at 4 °C overnight. Lymphocytes were incubated in ELISPOT plates for 16 h (37 °C, 5 % CO_2). IgG^+ MBCs were detected with 1 μg/ml biotinylated anti-IgG monoclonal antibody (MabTech) for 2 h at room temperature followed by Streptavidin-HRP (1:1000) (MabTech). Spots were developed with 3,3′,5,5′-tetrame

thylbenzidine (TMB) ELISPOT substrate (MabTech). The plates were counted using an ELISPOT reader (Advanced Imaging Devices, Germany). Spike-specific spots were calculated as the mean of duplicate wells after subtraction of negative controls and presented as spot forming units per million PMBCs (SFU/10^6 PBMCs).

SARS-CoV-2 T-cell responses

Spike-specific interferon-γ (IFN-γ), interleukin 2 (IL-2), and double-positive IFN-γ^+, IL-2^+ cytokine-producing T-cells were quantified using an IFN-γ/IL-2 FluoroSpot kit (MabTech), as previously described [14].

T-cell receptor (TCR) variable beta chain sequencing

EDTA samples were collected 0, 3- and 6-weeks post-vaccination from all vaccinees for ultradeep immunosequencing of the human TCRβ chains complementarity determining region 3 (CDR3) using the immunoSEQ Assay (Adaptive Biotechnologies, Seattle, WA). Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCRβ CDR3 for further analysis as previously described [15].

Identifying SARS-CoV-2-associated TCRβ sequences

One-tailed Fisher's exact tests were performed on all unique TCRβ sequences comparing their presence in SARS-CoV-2 PCR-positive samples (n = 1954) with negative controls (n = 3903) generating a list of SARS-CoV-2-associated sequences which are exclusive to, or greatly enriched, in PCR-positive samples. Filtering was performed to remove potential false positives associated with cytomegalovirus (CMV) seropositivity or human leukocyte antigen (HLA) alleles in SARS-CoV-2 negative healthy populations [16]. SARS-CoV-2-associated sequences contains 8631 rearrangements.

We assigned subsets of our enhanced TCR sequences to spike, and non-spike antigens based on data from multiplexed antigen stimulation assays. 917 TCRs were assigned to the SARS-CoV-2 spike protein and 1564 to non-spike viral proteins. We inferred whether an enhanced sequence was a $CD4^+$ or $CD8^+$ T-cell by statistically associating each sequence to a Class II or Class I HLA. HLA associations are derived from a set of 657 SARS-CoV-2 positive individuals who have genotyped HLAs. We built a binary logistic regression classifier with L1 regularization to determine which HLA best predicts the observed distribution of a given enhanced sequence across all HLA-typed cases. The L1 regularization strength was tuned to yield a single non-zero coefficient, giving a single inferred HLA association for each enhanced sequence. The inferred HLA associations were validated against the subset of enhanced sequences which overlap with our multiplex antigen stimulation assays [15].

Spike-specific TCR clonal breadth and depth were calculated using the set of SARS-CoV-2-associated TCRβ sequences. Breadth is calculated as the number of unique SARS-CoV-2-associated rearrangements out of the total number of unique productive rearrangements, while depth accounts for the frequency of those rearrangements in the repertoire.

Statistical analysis

Data were plotted using Graphpad Prism (version 9, La Jolla, USA). Statistical analyses were performed in R (Version 4.1.2) using the packages nlme and emmeans. We used linear mixed-effect models to compare humoral and cellular responses after vaccination in younger and older adults over the 9-months and compared to infected individuals with adjustments for demographic and clinical data, and for subject variation with repeated measures. A single global test for all possible 2-way interaction terms was performed for each model to avoid multiple testing. The interaction term was included in the model only if $P \leq 0.01$ to lower the likelihood of a false positive result. The estimated effects of covariates are presented with 95 % confidence intervals. Statistical differences between pairs of group means were done by post-hoc tests using Bonferroni adjustment for multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

Demographics of study population

We prospectively enrolled elderly and younger adults after BNT162b2 vaccination (2 doses at 3-week intervals), consisting of 449 younger adults (median 38 years old (yo), range 23–69yo, 69 % female) and 100 elderly (median 86yo, range 70–99yo, 63 % female) (Fig. 1, Table 1). The elderly had more comorbidities (84 %), mainly chronic heart disease, than younger adults (12 %). Fifteen per cent of the elderly were taking immunosuppressive medication, most commonly prednisolone for chronic obstructive pulmonary disease or rheumatic disease, compared to 1 % of the younger adults. Blood samples were collected at baseline, at 3-weeks, 6-weeks, 5- and 9-months after the first vaccination (Fig. 2A) and all vaccinees were pre-vaccination seronegative by RBD ELISA.

BNT162b2 vaccination induces durable, but less robust humoral immunity in older adults

We have conducted a comprehensive analysis of the kinetics and durability of the humoral and cellular responses after BNT162b2 vaccination. Binding IgG titers were measured against recombinant spike protein by ELISA. The first vaccination elicited spike-specific IgG in both groups, although the elderly had significantly lower geometric mean titers (GMT) than the younger adults (GMT 1503 vs 9578, $P < 0.0001$) (Fig. 2B). The second vaccination boosted spike-specific IgG titers in all vaccinees with the highest titers observed in younger adults, however, the elderly had a higher fold increase after the second dose (mean fold increase 30 vs 7). In addition to age, a mixed-effects model showed that IgG titers were also significantly lower for men than women and for vaccinees with comorbidities (Table S1). The elderly had significantly lower spike-specific IgG titers compared to the younger adults at 5- and 9-months (GMT 5-months 4048 vs 14713, 9-months 1483 vs 2724, $P < 0.0001$) (Fig. 2B), also when immunosuppressed individuals were excluded (Fig. S1). We used linear regression models to calculate the half-life of the spike-specific IgG response from peak levels measured after the second dose. We found that the geometric mean of the estimated IgG half-life was 3.1 months for the elderly compared to 3.6 months for the younger adults.

We studied the neutralizing antibody response after vaccination in the elderly and a representative subgroup of younger adults (Fig. 2C). Neutralizing titers were lower in the elderly than the younger adults after the first dose (GMT 16 vs 29), where only 32 % of the elderly had detectable (≥20) neutralizing antibodies compared to 71 % of younger adults. The second dose boosted neutralizing titers for both groups, with 92 % of the elderly having neutralizing titer ≥ 20 (GMT 115) compared to 100 % of the younger adults (GMT 469). Although neutralizing antibodies waned in both groups, titers were detectable in 100 % of younger adults (GMT 142) and 82 % of elderly (GMT 45) after 5-months. At 9-months

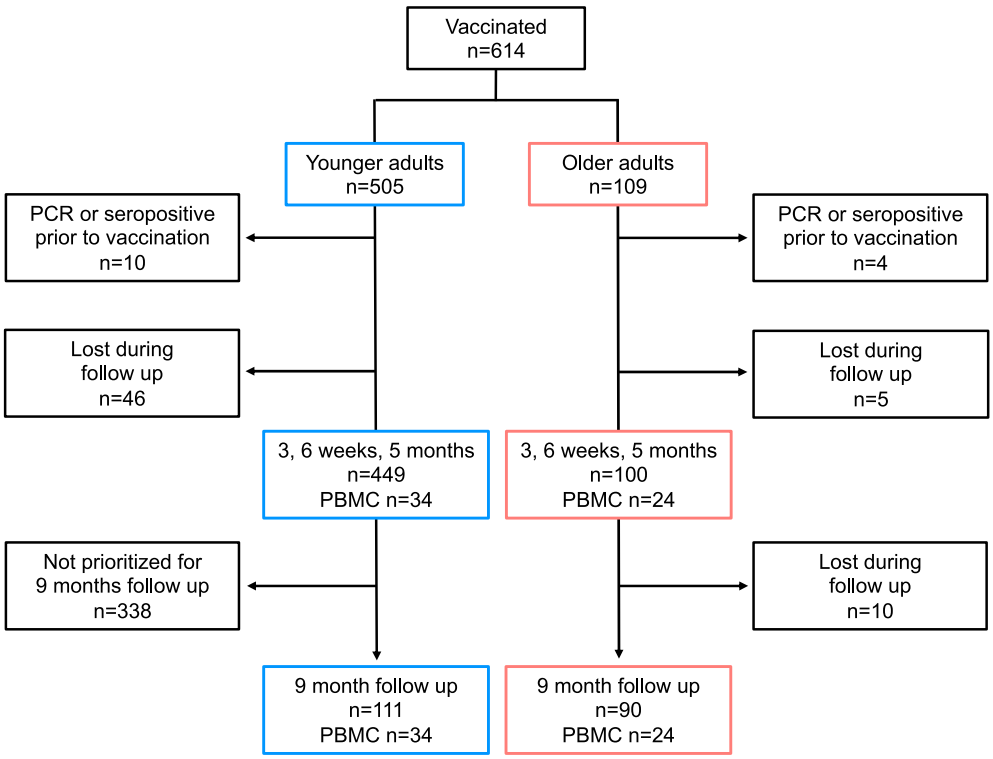

Fig. 1. Study population flowchart. Number of vaccinees eligible for inclusion at baseline and sampling of serum and peripheral blood mononuclear cell (PBMC) for the participants during the study.

Table 1
Demographics of the study population.

Subgroup	Younger adults, n (%)			Older adults, n (%)		Infected, n (%)	
	All[1]	B- and T-cells[2]	Subgroup 9 months	All[2]	B- and T-cells[2]	All[3]	B- and T-cells[4]
Total number of subjects	449	34	111	100	24	183	68
Female/male	309/140 (69/31)	22/13 (63/37)	75/36 (66/34)	63/37 (63/37)	15/9 (62/38)	95/88 (52/48)	33/35 (5149)
Median age	38	39	41	86	86	47	45
[range]	[23–69]	[27–63]	[23–66]	[70–99]	[73–93]	[23–80]	[16–80]
Comorbidity[5]	54 (12)	2 (6)	13 (12)	84 (84)	21 (88)	74 (40)	27 (40)
Immunosuppression[6]	5 (1)	1 (3)	3 (3)	15 (15)	7 (29)	5 (3)	2 (3)

[1] Sampled on day 0, 3- and 6-weeks, 5-months.
[2] Sampled on day 0, 3- and 6-weeks, 5-months, 9-months.
[3] Sampled at 2-, 6- and 12-months after diagnosis.
[4] Sampled at 6-months (B-cells n = 10, T-cells n = 38) and 12 months (B- and T-cells n = 63).
[5] Comorbidities include chronic heart disease, chronic lung disease, chronic liver disease, chronic kidney disease, diabetes, cancer, rheumatic disease, neurological disease, autoimmune disease.
[6] Inherent immunosuppressive disease, HIV, organ transplant, chemotherapy, Prednisone or other immunosuppressive medication.

post-vaccination, 95 % of younger adults still had detectable neutralizing antibodies (GMT 57), whereas the percentage of elderly had decreased to 58 % (GMT 24). We estimated the half-life and time for the neutralizing titers to fall below the level of detection (<20). The geometric mean of the estimated half-life of neutralizing titers was comparable for the younger adults (4.0 months) and the elderly (4.3 months). However, the estimated duration of detectable neutralizing titers was 12.5 months for the younger adults and only 6.2 months in the elderly (Fig. S2). Similarly to spike-specific IgG, the linear mixed-effects model analysis showed that the neutralizing antibodies were also lower for the elderly and for men (Table S1).

PBMC were collected from a subgroup of 34 younger adults and 24 elderly to investigate the spike-specific memory B-cell (MBC) response by ELISpot after the first and second dose, and long-term after 5- and 9-months (Table 1, Fig. 2D). The first dose induced significantly higher levels of MBCs in the younger adults compared to the elderly ($P < 0.05$). After the first dose, 87 % of younger adults had detectable spike-specific MBCs (mean SFU 329, range 0–790), whereas MBCs were only detected in 38 % of the elderly (mean SFU 68, range 0–440). The second vaccination boosted the number of MBCs in the elderly (mean SFU 198, range 0–1560), although four elderly subjects were non-responders (17 %), two of whom were taking immunosuppressive medication. In contrast, we found that the second dose induced more limited boosting of MBCs in the younger adults (mean SFU 348, range 0–1030), where two younger adults (6 %) had no increase in MBCs. The MBCs were generally maintained after 5- and 9-months post-vaccination or increased moderately in younger adults. The frequency of MBCs in the elderly increased to mean SFU 228 (range 0–1240) after 5-months and mean SFU 251 (range 0–870) after 9-months, although this increase was not significant. Similarly, the frequency of MBCs in younger adults increased moderately to mean SFU 456 (range 10–2000)

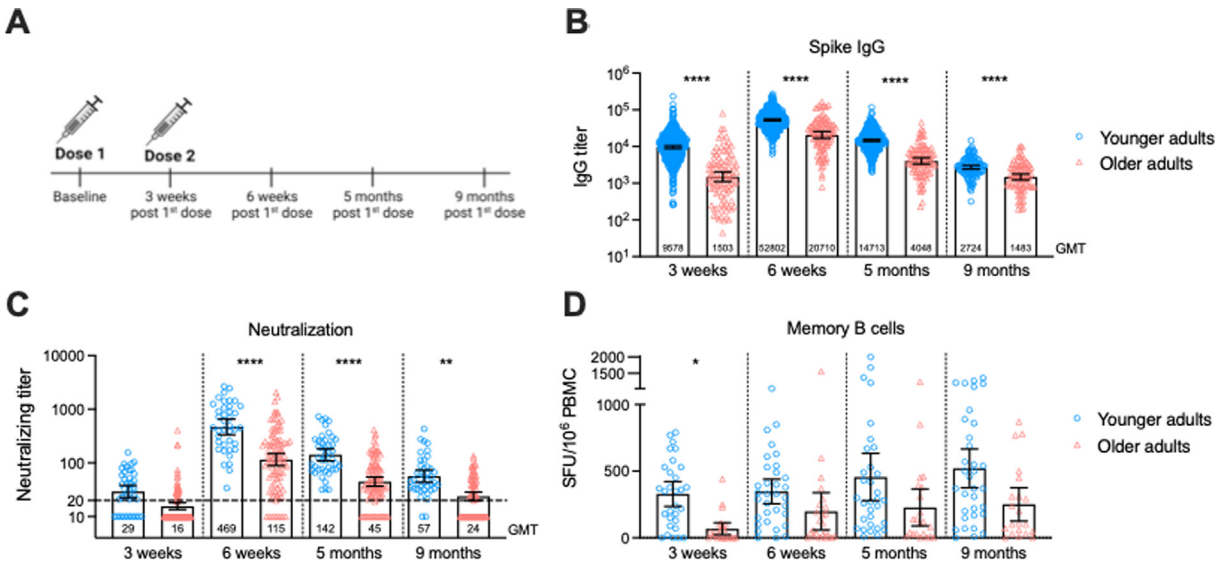

Fig. 2. Durability of spike-specific humoral immune responses after BNT162b2 vaccination. (A) Study design showing time points for vaccination and blood sampling in young adults (blue circles) aged 23–69 years (n = 449) and elderly persons (red triangles) aged 70–99 years (n = 100). Each symbol represents one individual. (B) Anti-spike serum IgG endpoint titers measured by ELISA. Data are presented as geometric mean titer (GMT) with 95 % confidence intervals (CIs). (C) Neutralizing antibody responses measured by microneutralization assay against the ancestral D614G strain in all elderly and a subgroup of adults including those that provided peripheral blood mononuclear cells (PBMCs) (n = 41). The neutralizing titer was defined as the reciprocal serum dilution resulting in 50 % neutralization. The data are presented as GMT with 95 % CIs. (D) Spike-specific memory B-cell responses were measured by ELISpot using PBMCs collected from a subgroup of vaccinated younger adults (n = 35) and elderly (n = 24). The frequency of spike-specific memory B-cells were defined as spot forming units (SFU) per 10^6 PBMC. The data are presented as mean with 95 % CIs. Mixed-effects model with normalized outcome variables with fixed effects of sex, age group, presence of comorbidity, use of immunosuppressive medication and age-by-time interaction (except neutralization and memory B-cells), and individual repeated measures as a random factor. Significance of differences between pairs of group means was assessed by post-hoc tests. P values were only reported if they were significant at the 5 % level after Bonferroni correction. ****P < 0.0001, **P < 0.01, *P < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

after 5-months and mean SFU 521 (range 0–1360) after 9-months.

BNT162b2 vaccination expands the spike-specific T-cell response

The immunoSEQ assay is a high throughput ultra-deep sequence-based method to quantify spike-specific T-cell responses after natural infection or vaccination [17]. This method allowed us to assess the breadth and depth of the spike-specific TCR repertoire for all vaccinees at baseline and after the first and second vaccination. Clonal breadth was defined as the relative number of distinct spike-specific T-cell clonotypes as a fraction of the overall repertoire, and clonal depth as the extent of expansion of spike-specific T-cells.

The spike-specific TCR breadth and depth increased in both groups after the first and second vaccination, demonstrating expansion of spike-specific T-cells (Fig. 3). The younger adults had significantly higher TCR breadth (Fig. 3A) and depth (Fig. 3B) compared to the elderly after the first and the second vaccination, indicating that the elderly had fewer expanded T-cells and a narrower spike-specific TCR repertoire (Table S2). The younger adults had the highest fold increase in spike-specific TCR breadth after the first vaccination (mean fold increase 11 vs 4), while the fold increase was comparable between the groups after the second dose (mean fold increase 1 vs 2). Overall, both the breadth and depth of the spike-specific TCR repertoire was highest for the human leukocyte antigen (HLA) class II-associated T-cells, indicating that $CD4^+$ T-cells made up the largest proportion of the vaccine-induced T-cell response.

To complement the TCR data, we analyzed the functionality of the spike-specific T-cell response using PBMCs in the FluoroSpot assay in a subgroup of younger adults (n = 34) and elderly (n = 24) (Fig. 4, Table 1). Single cytokine-producing (IFN-γ or IL-2) T-cells (Fig. 4A–B) and double cytokine-producing IFN-γ^+, IL-2^+ T-cells (Fig. 4C) were measured using overlapping peptide pools from the spike protein. Spike-specific TCR breadth and depth correlated with the number of functional spike-specific T-cells measured by FluoroSpot (R = 0.462 and R = 0.375 respectively after 1st vaccination and R = 0.583 and R = 0.522 respectively after 2nd vaccination) (Fig. S2). The first vaccination increased IFN-γ and IL-2 single-producing and double-producing T-cells from baseline levels in both groups, with all but one younger adult and two elderly responding. The magnitude of the response was higher in the younger adults than the elderly after the first dose, although not significant. The second vaccination boosted T-cell responses in both groups, with 96 % (23/24) responders among the elderly and 100 % (34/34) responders among the younger adults. No significant difference in the T-cell responses were observed between the younger adults and the elderly, although a trend towards higher frequencies was consistently observed in the younger adults. The spike-specific T-cells gradually declined from peak levels after the second dose in both groups. Low frequencies of T-cells were detected at 9-months in both vaccine groups, with no significant differences between the groups. The proportion of single cytokine-producing T-cells secreting IFN-γ or IL-2 was similar after 3-weeks in both groups but became skewed more towards IFN-γ over time. This transition was faster in the younger adults than the elderly (Fig. 4D). The proportion of IFN-γ and IL-2 double-producing T-cells was always lower than single cytokine producing cells for both groups at all timepoints.

Age-dependent variation in spike-specific immune responses induced by SARS-CoV-2 infection and vaccination

We then compared the magnitude and durability of spike-specific immune responses induced by vaccination and SARS-CoV-2 infection. Home-isolated, naturally infected participants were prospectively recruited in March and April 2020 during the

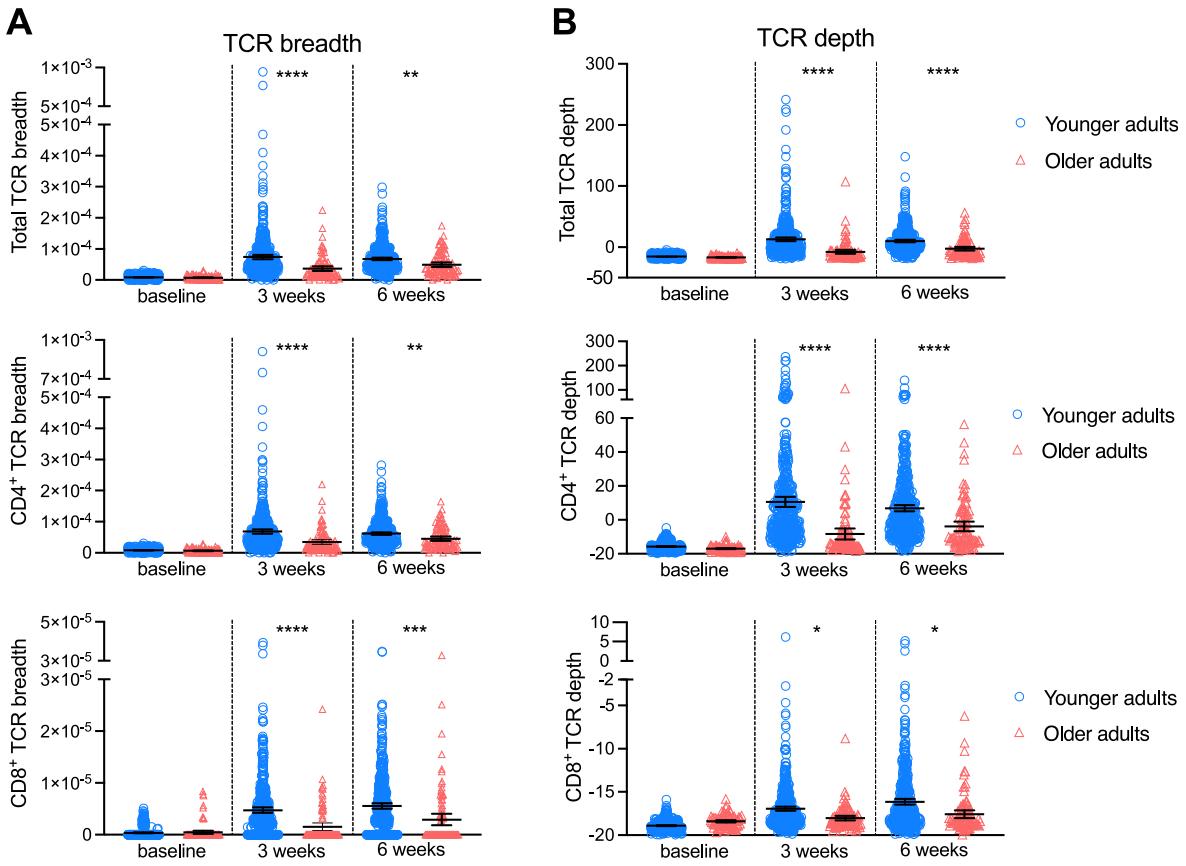

Fig. 3. Spike-specific T-cell receptor sequencing after BNT162b2 vaccination. (A) The clonal breadth of the spike-specific T-cell receptor (TCR) repertoire measured by immunoSEQ in vaccinated adults (blue circles) aged 23–69 years (n = 449) and elderly persons (red triangles) aged 70–99 years (n = 100). Blood samples were tested at baseline, and 3 weeks after first and second vaccination (6 weeks post-first vaccination). Each symbol represents one individual. The proportion of unique spike-specific TCR sequences (breadth) for all T-cells (top), $CD4^+$ T-cells (middle) and $CD8^+$ T-cells (bottom). Data are presented as mean with 95 % confidence intervals. (B) The frequency of unique spike-specific TCR sequences (depth) for the total TCR repertoire (top), $CD4^+$ T-cells (middle) and $CD8^+$ T-cells (bottom). Mixed-effects model with normalized outcome variables with fixed effects of sex, age group, presence of comorbidity, use of immunosuppressive medication and age-by-time interaction (except $CD8^+$ TCR depth), and individual repeated measures as a random factor. Significance of differences between pairs of group means was assessed by post-hoc tests. P values were only reported if they were significant at the 5 % level after Bonferroni correction. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

first pandemic wave [12]. The infected group experienced mild-to-moderate COVID-19 and consisted of 183 subjects (median age 47 yo, range 23–80 yo, 52 % female and 48 % male), where 40 % had comorbidities and 3 % were taking immunosuppressive medication (Table 1). Immune responses were measured 8-weeks, 6- and 12-months after SARS-CoV-2-confirmed diagnosis and were compared with responses measured at 6-weeks, 5- and 9-months after first vaccination, respectively.

The second dose boosted spike-specific IgG to higher peak levels at 6-weeks in both vaccine groups than the infected group 8-weeks post-infection. Vaccinees had significantly higher IgG titers at 5-months compared to the infected patients after 6-months, although only the younger adults remained significantly higher than the infected group after 9-months (Fig. 5A). Furthermore, the younger adults had significantly higher peak neutralizing titers at 6-weeks while the infected group had significantly higher neutralizing titers after 12-months compared to the vaccinated elderly at 9-months.

The durability of the spike-specific memory B and T-cell responses were compared after vaccination and infection in a subgroup of individuals (Table 1). There was a non-significant trend of higher frequencies of MBCs in the infected group at 12 months compared to the vaccinees at 9 months, with lower responses consistently observed in the vaccinated elderly although only significantly lower than the infected at 5–6 months (Fig. 5A). Durable single cytokine-producing $IL\text{-}2^+$ and double cytokine-producing $IFN\text{-}\gamma^+$, $IL\text{-}2^+$ T-cells were significantly higher 12-months post-infection compared to 9-months post-vaccination in elderly and younger adults (Fig. 5B). Whereas, single cytokine-producing $IFN\text{-}\gamma^+$ T-cells were significantly higher in the infected group at 6–12 months compared to only the elderly vaccinees after 5–9 months. The radar chart shows that the infected group maintained humoral and cellular responses between 6- and 12-months, whereas this was not observed in either of the vaccine groups between 5- and 9-months post-vaccination with the exception of MBCs (Fig. 5C).

We further compared the age-specific magnitude and durability of spike-specific antibody responses following vaccination and infection. There were notable differences in the magnitude and durability of IgG and neutralizing titers following vaccination and infection for all age groups. We found that younger vaccinated subjects (23–55 yo) had significantly higher IgG and neutralizing titers than infected individuals of the same age during peak levels (6 vs 8 weeks post-infection) (Fig. 6A, D, Table S3). The difference in IgG and neutralizing titers between younger infected and vaccinated individuals decreased at 5-months (Fig. 6B, E), with no significant differences between infected and vaccinated individuals aged 40–69 yo observed by 9-months (Fig. 6C, F). In contrast, the infected older individuals (70–99 yo) had significantly higher peak neutralizing titers, although similar peak IgG titers, than the vaccinees of

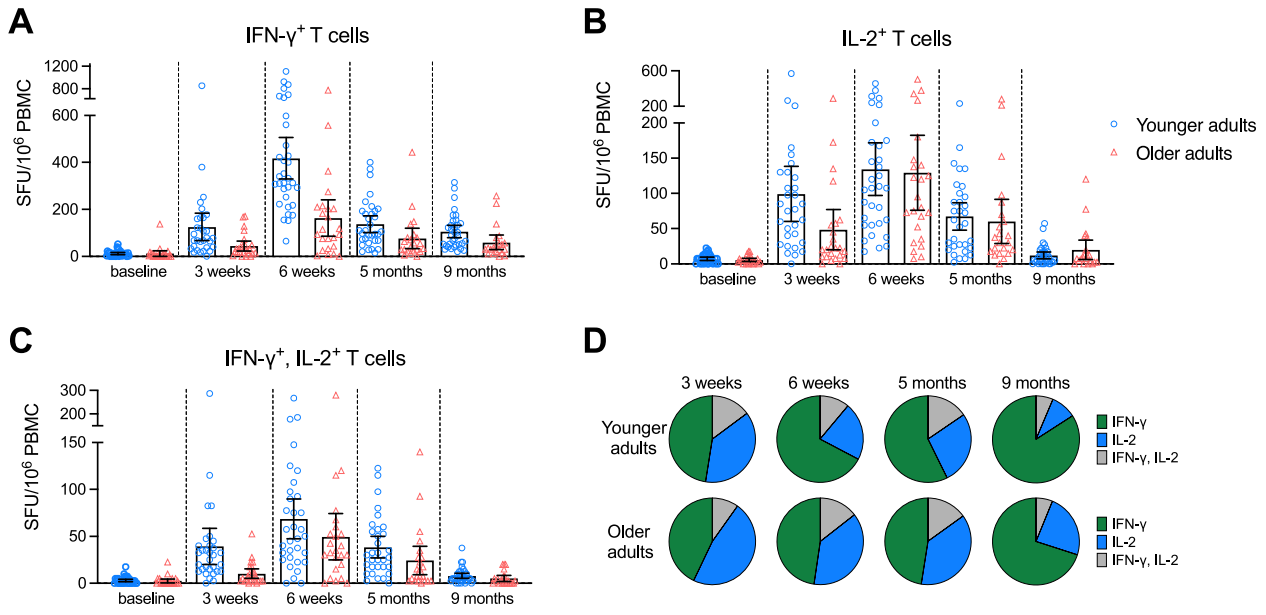

Fig. 4. Functional spike-specific T-cell responses after BNT162b2 vaccination. (A–C) The frequency of interferon-γ (IFN-γ) (A), interleukin-2 (IL-2) (B) and double cytokine-producing spike-specific T-cell responses (C) measured by fluorospot in vaccinated adults (n = 35, blue circles) and elderly (n = 24, red triangles). Each symbol represents one individual. T-cell frequencies are reported as spot-forming units (SFU) per 10^6 peripheral blood mononuclear cells at baseline, and 3 weeks after first and second vaccination (6 weeks post-first vaccination) and 5- and 9-months post-first vaccination. Data are presented as mean with 95 % confidence intervals. (D) The proportion of T-cells producing single cytokines IFN-γ (green) or IL-2 (blue), or double-producing IFN-γ and IL-2 (gray) in adults (top row) and elderly (bottom row). The proportions are presented as percentage of single or double-producing T-cells of the total number of T-cells measured in the younger (n = 35) and older adults (n = 24). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the same age group. These infected 70–99 yo individuals also had higher and more durable IgG and neutralizing titers at 12-months post-infection compared to vaccinated subjects at 9-months (Fig. 6C, F).

We compared the geometric mean of estimated time for neutralizing titers to fall below the level of detection (neutralizing titer < 20) for infected and vaccinated subjects. Our calculation estimated that infected subjects aged 70–99 yo (n = 15) would have detectable neutralizing antibodies for up to 14-months compared to only 6-months for vaccinated elderly (n = 93), demonstrating increased durability after infection for the oldest individuals. This disparity was largest for subjects aged 56–69 yo where the vaccinees (n = 13) were estimated to have detectable neutralizing antibodies for 15-months compared to 28-months in the infected subjects (n = 36). The durability of neutralizing antibody titers was more comparable for the two youngest age groups 23–39 yo (vaccinees 12 months, n = 20 vs infected 12 months, n = 72) and 40–55 yo (vaccinees 15 months, n = 13 vs infected 17 months, n = 65).

In summary, infected individuals had more durable spike-specific humoral and cellular immune responses at all ages, but particularly antibody responses in older infected individuals.

Discussion

The rapid development and implementation of COVID-19 vaccines have changed the trajectory of the COVID-19 pandemic. Vaccination is the best method for protection against severe disease and long-term sequalae. There is limited data on the longevity of immune responses exceeding 6-months post-vaccination, especially among elderly persons who are at high risk of severe COVID-19, which we aimed to address in this study. Here, we have comprehensively investigated the kinetics and durability of humoral and cellular immune responses in younger and old adults following two doses of BNT162b2 vaccination for up to 9-months and compared this to naturally infected home-isolated individuals.

Overall, we found that home-dwelling old adults had markedly lower spike IgG and neutralizing antibody responses compared to younger adult vaccinees, particularly 3-weeks after the first and second vaccinations. At 9-months, both younger and older adults had low neutralizing antibody titers, with only 58 % of the elderly having detectable neutralizing antibodies compared to 95 % in the younger adults. COVID-19 mRNA vaccines have been found to induce a persistent germinal center reaction in young adults leading to durable antibody responses [18]. Immunosenescence is commonly observed in older persons and has been associated with lower humoral and cellular immunity following SARS-CoV-2 vaccination [8,19–21]. A diminished germinal center response has been identified as a key contributing factor of poor humoral immunity in elderly [19], which could explain the lower magnitude and hence durability of the antibody response. Although antibody levels wane over time, MBCs are detectable after mRNA vaccination and have been reported to increase between 3 and 6-months, and also recognize Delta and Omicron VOC [20,22,23]. Here we have extended these findings to show that MBCs continue to increase up to 5-months post-vaccination in the elderly and up to 9-months in younger adult vaccinees. MBCs are an important part of durable protection and two doses of BNT162b2 vaccine induce persistent MBCs that rapidly respond to infection and produce neutralizing antibodies to limit the infection, also local IgA in the saliva [24]. Furthermore, a third booster vaccination results in expansion of MBCs that produce more potent and broadly reactive antibodies [25]. We found that MBCs also persisted in the elderly for up to 9-months, likely contributing to protection and immunogenicity of future booster vaccinations for this high-risk group.

Older individuals are at significantly higher risk of severe disease and death from COVID-19 [4,5], thus optimal immunogenicity of vaccines is of great importance for this group. Longer spacing between priming and booster doses could be beneficial for vaccine distribution during a pandemic, where vaccine supply is limited.

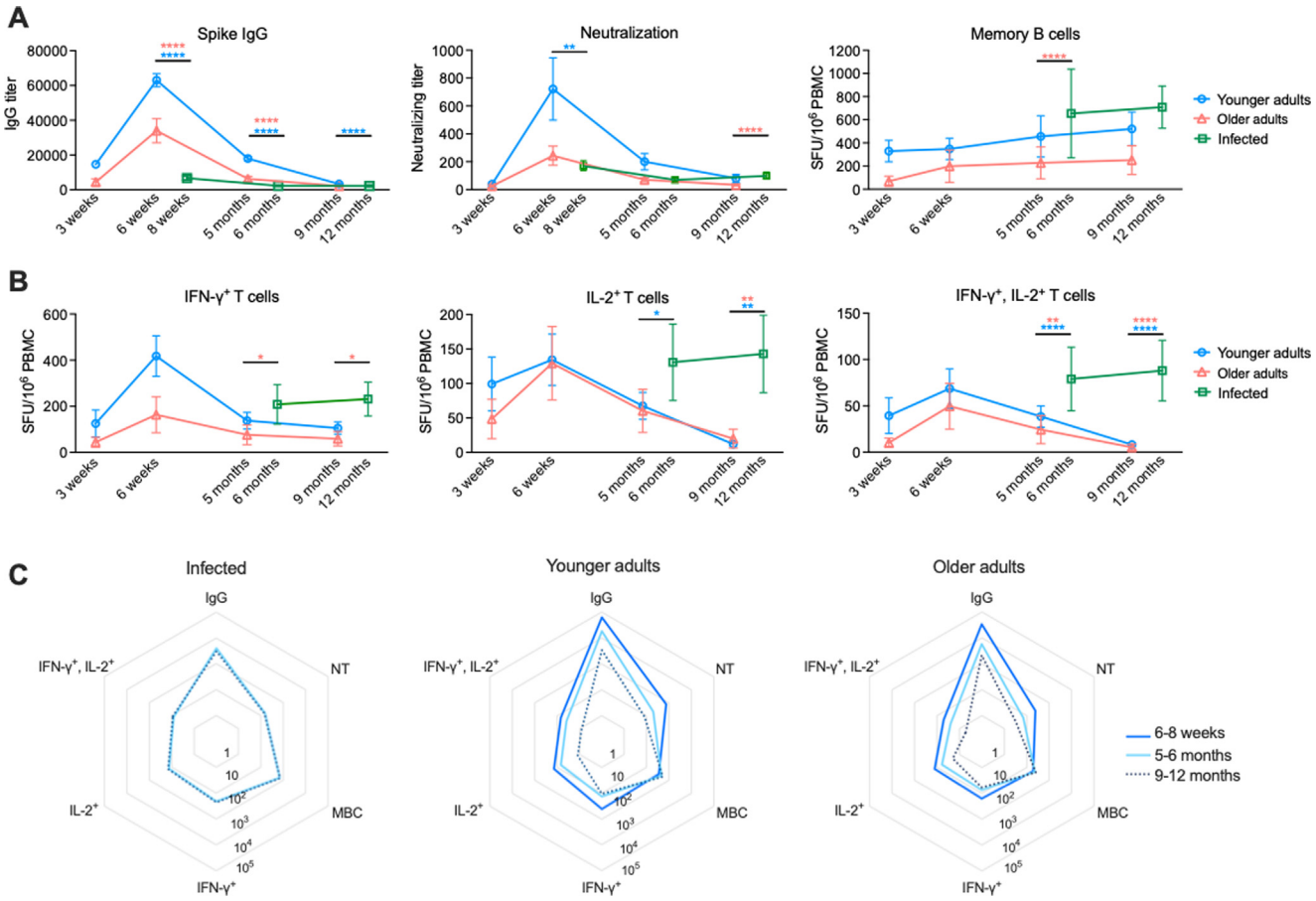

Fig. 5. Spike-specific immune responses after vaccination and SARS-CoV-2 infection. (A) Spike-specific IgG titers, neutralizing titers and spike-specific memory B-cell responses were measured for vaccinees 3 and 6 week, and 5- and 9-months post-vaccination and infected subjects at 8 weeks, 6- and 12-months post-infection. Spike-specific IgG and neutralizing titers are presented as geometric means with 95 % confidence intervals (CIs). Spike-specific memory B-cell responses were measured by ELISpot using peripheral blood mononuclear cells (PBMCs). The frequency of spike-specific memory B-cells were defined as spot forming units (SFU) per 10^6 PBMC. The data are presented as the mean with 95 % CIs. Statistical significance was assessed between the infected group (green) and vaccinated older (red) or younger adults (blue) using a mixed effects model for normalized outcome measures, adjusted for repeated-measure subject variance and demographic factors, and post-hoc tests with Bonferroni correction. Red stars represent the significance level between the older adults and the infected group and blue stars represent significance level between the younger adults and the infected group. IgG titer: younger adults n = 449, older adults n = 100, infected n = 198. Neutralizing titer: younger adults n = 43, older adults n = 100. Memory B-cells: younger adults n = 34, older adults n = 24, infected 6 months n = 10, infected 12 months n = 63. (B) Spike-specific T-cell responses measured as the frequency of interferon-γ (IFN-γ), interleukin-2 (IL-2) and double cytokine-producing spike-specific T-cells measured by FluoroSpot assay in vaccinees at 3- and 6-week, and 5- and 9-months post-vaccination (younger adults n = 35, older adults n = 24) and infected subjects at 6 and 12 months post-infection (6 months n = 38, 12 months n = 63). Data are reported as mean SFU/10^6 PBMC with 95 % CIs. (C) Radar chart summarizing durable spike-specific immune responses in SARS-CoV-2 infected (left) and vaccinated younger adults (middle) and older adults (right). The data are presented as means on a log-axis. NT (neutralizing titer), memory B-cell (MBC). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The original licensure trials used a 3-week interval between the first and second dose [2], however, longer intervals have been associated with improved vaccine immunogenicity [26,27]. Our study showed that the kinetics of homologous humoral immunity differed between older and younger adults. We found that younger adults had robust antibody responses after the first dose and had moderate boosting of MBCs after the second dose, as previously observed [20,23], indicating that a prolonged interval is acceptable for this group. In contrast, the first dose was markedly less immunogenic in the elderly who had superior boosting of spike-specific IgG titers and MBCs after the second dose compared to the younger adults. Our data demonstrates the necessity of a short 3-week interval between first and second vaccine doses for the elderly to provide optimal protection in a pandemic setting. Another important aspect to inform public health responses and vaccine deployment is the durability of the vaccine induced immunity in different age groups. We calculated the estimated half-life for spike-specific IgG and neutralizing titers and found that the rate of waning was comparable between the younger and older adults. However, the older vaccinees had significantly lower magnitude of IgG and neutralizing titers compared to the younger adults at all time points after vaccination, which may impact the durability of protection. Detectable neutralizing titers were estimated to last twice as long in the younger adults than the elderly after vaccination. Interestingly, natural infection induced more durable neutralizing titers estimated to last 8-months longer than vaccination among the elderly. Real world effectiveness studies show reduced protection from infection and symptomatic disease 6-months after vaccination, with older adults having the greatest reduction [28]. These findings suggest that SARS-CoV-2 naive individuals over 70 years would benefit from a third booster dose by 5-months after the first vaccination. Overall, our data provide valuable insights into the kinetics of the antibody response in the elderly and may have implications for vaccine regimens and distribution in the future.

Emerging data have demonstrated the importance of T-cells in reducing infection and disease severity [29]. IL-2 is a cytokine primarily produced by activated $CD4^+$ T-cells and it is essential for T-

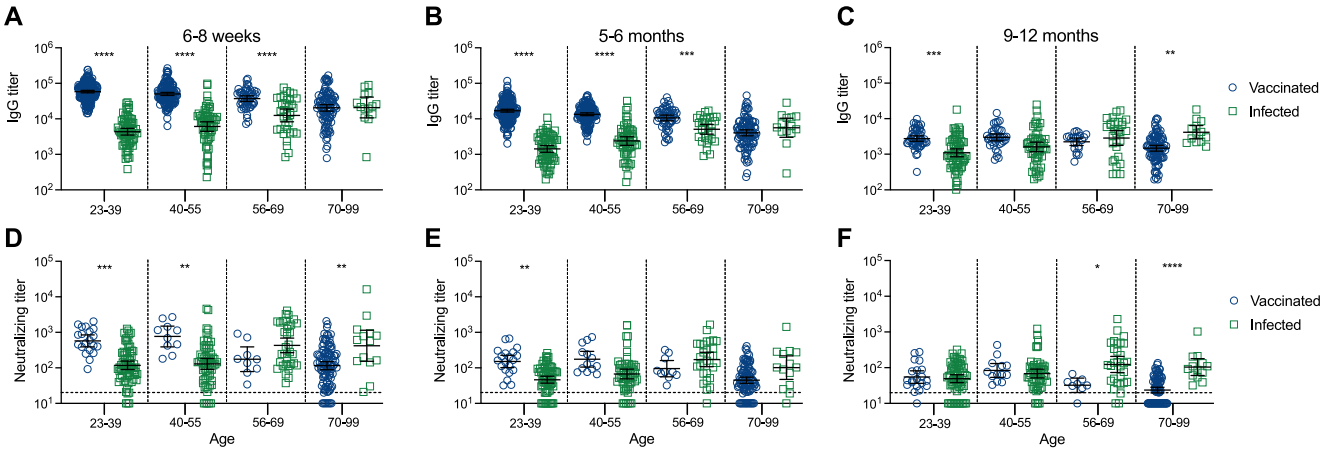

Fig. 6. Age-specific antibody responses after vaccination and natural SARS-CoV-2 infection. (A–F) Spike-specific IgG (A–C) and neutralizing titers (D–F) in vaccinated individuals (n = 549) (dark blue circles) and infected individuals (n = 183) (green squares) were divided into 15-year age groups. Blood samples were collected 6-weeks after the first vaccination (3-weeks after the second vaccination), 5- and 9-months post-vaccination. Home-isolated SARS-CoV-2 infected individuals (n = 183) provided blood samples 8-weeks, 6- and 12-months post-acute infection. Data are presented as geometric mean titer (GMT) with 95 % confidence interval (CI) and each symbol represents one individual. The threshold for detectable neutralizing antibodies (<20) is indicated with a dotted line. Multiple linear regression analysis was used to test if vaccination or infection group predicted log normalized IgG or neutralizing titers, adjusted for repeated-measure subject variance and covariates sex, age, comorbidity, use of immunosuppressive medication and age-by-group interaction. P values were only reported if they were significant at the 5 % level after Bonferroni correction. ****P < 0.0001, ***P < 0.001, **P < 0.01, *P < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cell survival and differentiation, whilst IFN-γ is important for modulating the adaptive immune response and for clearance of viral pathogens. The frequencies of the spike-specific single and double IFN-γ and IL-2 producing T-cells were higher in the younger adults than the elderly at early time points after vaccination, although not significant, which is in agreement with previous findings [8]. Single IL-2$^+$ and double IFN-γ$^+$, IL-2$^+$ T-cell responses were maintained at a significantly higher level one year after infection than in vaccinees, irrespective of age. Future studies to investigate hybrid immunity after infection and vaccination are needed for the elderly.

The immunoSEQ assay is a sequence-based method based on advanced statistical methods and machine learning to identify and quantify spike-specific T-cell responses after natural infection or vaccination. Our study demonstrates how recent developments in understanding of the unique rearrangements (breadth) and frequency of unique TCRs (depth) allows for high-throughput evaluation of spike-specific T-cell responses in large cohorts after vaccination. Lower TCR diversity against SARS-CoV-2 epitopes has been associated with severe COVID-19 [30,31], demonstrating the value of broader TCR repertoires. We found that BNT162b2 vaccination increased the breadth and depth of the spike-specific CD4$^+$ and CD8$^+$ TCR repertoire in both younger and older adults, with the second vaccination being particularly important in the elderly. Others have shown that mRNA vaccination induces durable polyfunctional CD4$^+$ and CD8$^+$ T-cell responses with a stem cell memory phenotype [32]. Our results show that one dose of mRNA BNT162b2 vaccine elicited broader spike-specific T-cell responses, compared to previous reports of one dose of Ad26. COV2.S vaccine [33]. Importantly, vaccine-induced spike-specific T-cell responses are not severely impacted by SARS-CoV-2 VOC [33,34] and Omicron is predicted to have 20–30 % reduction in CD4$^+$ and CD8$^+$ spike-specific T-cell responses [35]. Therefore, T-cells may still provide some degree of protection severe disease and more so after booster or bivalent COVID vaccination.

Strengths of our study include the comprehensive immunological comparison over time of vaccinated and infected cohorts, and inclusion of the oldest vaccinees. Although the elderly subjects in our study were home-dwelling with a number of comorbidities, they may have been healthier than similar age groups in other geographical areas, as Norway has a high life expectancy. Although the infected individuals experienced mild-to-moderate COVID-19 and did not require hospitalization, we cannot determine if age and disease severity independently influence immune responses. This study focused on spike-specific immune responses, but infected individuals also have antibody and cross-reactive T-cell responses to the more conserved nucleocapsid and membrane proteins [36]. Furthermore, a caveat of this study is the number of subjects providing PBMC, which limits our assessment of MBC and T-cell responses.

Our data provide valuable information on the kinetics and durability of SARS-CoV-2 immune responses for younger adults and an understudied, high-risk, elderly group. Of importance is our finding of more durable binding and neutralizing antibody levels 12-months after natural infection compared to 9-months after vaccination, even in older individuals 70–99 years old. Likewise, spike-specific MBC and T-cell responses were maintained one year after infection, particularly evident for T-cell responses, which waned after vaccination but were maintained after infection. Although older adults had consistently lower spike-specific immune responses after vaccination compared to younger adults, the elderly had more robust and durable antibody responses after infection. Our results indicate that infection induces more robust and durable immune responses in the elderly, which is not achieved by two doses of BNT162b2 vaccine. It is important to emphasize that the persistence of symptoms and complications after COVID-19 are well-documented and vaccine-induced immunity is therefore the best and safest way to acquire immunity to SARS-CoV-2 [37,38]. However, our study suggests that SARS-CoV-2 immune responses induced by vaccination and infection are inherently different in young and old adults. Further studies to investigate how age affects the immune responses induced by infection and vaccination are needed, and have the potential to inform the rationale design of vaccines for older adults.

Data availability

All data are available in the main text or the supplementary materials.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ian Kaplan, Heidi Chapman, Rebecca Elyanow, Shahin Shafiani are employed by Adaptive Biotechnologies.

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Author contributions

RJC, NL, KAB and KGIM conceptualized the study. LH, KAB, AB, FZ, GB, TBO, AM, JSO, MCT performed experiments. RE, SS, HC, IK performed the TCR sequencing and data analysis. HHS, NUE, EBF, MS, HS, JSO, CT, KIGM performed the clinical studies. LH visualized and managed the data. AB performed the statistical analyses. LH, RJC, NL wrote the original draft and all authors reviewed and edited draft.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jvacx.2023.100262.

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