

Differential vaccine-induced kinetics of humoral and cellular immune responses in SARS-CoV-2 naive and convalescent health care workers

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Abstract

Effective vaccination is a key element in the exit strategy from the current severe acute respiratory syndrome- CoV coronavirus-2 (SARS-CoV-2) pandemic, and may also offer protection against severe disease from future variants of concern. Here we prospectively monitored T- cell responses over time, using ELISpot interferon- γ (INF- γ) release assays, and B- cell responses, using serological tests, after vaccination and booster with BioNTech/Pfizer mRNA (Pfizer) and Janssen vector (Janssen/Johnson & Johnson) vaccines in hospital health care workers. Vaccine recipients were divided into seropositive and seronegative individuals at baseline, in order to determine the effect of natural immunity on vaccine-induced immune kinetics.

We found that convalescent individuals mounted higher spike-specific INF- γ -secreting T cell responses and B- cell-mediated IgG responses, after receiving the Janssen vaccine or the first dose of the Pfizer vaccine. IgG levels corresponded to the virus neutralisation capacity as measured by VNT assay. At 8 months post vaccination, spike-specific cellular immunity waned to low levels in individuals with or without prior natural immunity, whereas waning of humoral immunity occurred predominantly in naive individuals. The booster shot effectively re-induced both cellular and humoral immune responses.

To conclude, our data supports the implemented single-dose mRNA booster strategy employed in the Netherlands. Furthermore, the level of pre-existing natural immunity may be factored into determining the optimal time window between future booster vaccines.

Key words: SARS-CoV-2, mRNA vaccine, vector vaccine, ELISpot IFN- γ release assay, T-cell response, ELISA

Background

The development of the humoral and cellular immune response generated by primary SARS-CoV-2 infection has been the focus of an intense research effort over the past year (Dan *et al.* 2021; den Hartog *et al.* 2021; Zollner *et al.* 2021). The effectiveness of mRNA and vector vaccine-induced humoral responses in immunologically naïve individuals has been well established (Polack *et al.* 2020; Sadoff *et al.* 2021), including the protection gained by booster vaccination (Bar-On *et al.* 2021; Sablerolles *et al.* 2022). For development of future vaccination booster strategies, it is important to consider the potential role of past SARS-CoV-2 infection on the kinetics of B- and T- cell responses following vaccination. Despite research on vaccine-induced antibodies and their ability to neutralise virus particles, longitudinal data on simultaneously assessed B- and T- cell responses in SARS-CoV-2 naïve and immune individuals is limited (GeurtsvanKessel *et al.* 2022).

Recently, we demonstrated that T-cell responses in COVID-19 patients can readily be detected by using an enzyme-linked immunosorbent spot (ELISpot) interferon- γ release assay (Thijssen *et al.* 2020). T-cell responses against peptide pools of SARS-CoV-2 membrane, nucleocapsid and spike proteins showed the highest discriminative power to detect SARS-CoV-2 immunity.

Here, we examined T- and B- cell responses in parallel using interferon- γ release ELISpots, anti-spike IgG ELISA, and virus neutralisation assays in a well-defined cohort of Dutch hospital health care workers that received the BioNTech/Pfizer

mRNA (here referred to as Pfizer vaccine) or the Janssen/Johnson & Johnson vector vaccine (here referred to as Janssen vaccine). We compared the immune responses in HCW with and without serological evidence of passed SARS-CoV-2 infection, in order to investigate how natural immunity impacts vaccine-mediated cellular and humoral immune responses.

Methods

Ethics statement

The regional Medical Research Ethics Committees United approved the study (Nieuwegein, the Netherlands; MEC-U: NL73618.100.20). Informed consent was obtained from all participants. Statistical analysis was performed with GraphPad Prism 9.1 (GraphPad Software). T-cell responses against S and S1 peptide pools (SFC/ 2.5×10^5 PBMCs) between groups were tested using the 2-tailed Mann-Whitney test, with $P < 0.05$ considered significant.

Study subjects

All subjects were health care workers recruited from the medical laboratory and medical ward who had previously been enrolled in the SARS-CoV-2 Immune Response (SIR) study, conducted in November 2020 in the Diaconessenhuis Hospital Utrecht; a large teaching hospital in the Netherlands. Subjects were randomly contacted based on SARS-CoV-2 seropositive or seronegative status as determined in the SIR study.

Blood was drawn from 56 participants (SARS-CoV-2 seronegative $n=42$, SARS-CoV-2 seropositive $n=14$) prior to vaccination with the BioNTech/Pfizer mRNA (BNT162b2) vaccine and another 24 (SARS-CoV-2 seronegative $n=18$, SARS-CoV-2

seropositive n=6) prior to vaccination with Janssen/Johnson & Johnson (Ad26.COV2.S; JNJ-78436735) vaccine. At subsequent time points blood was drawn 2-3 weeks after individuals were fully vaccinated, prior to the booster shot 2-3 weeks thereafter. Baseline values were obtained from SIR study data. At each time-point, HCWs were asked to report history of symptoms or SARS-CoV-2 positive PCR tests / self-tests that may indicate occurrence of (re-)infections during the study follow-up.

Isolation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated exactly as reported previously from whole blood specimens which were collected in lithium heparin tubes (van Gorkom *et al.* 2020). PBMCs were counted by using the Sysmex Automated Hematology Analyzer XP-300 (Sysmex Nederland, Etten-Leur, Nederland) as was published previously (van Gorkom *et al.* 2020).

In-house SARS-CoV-2 ELISpot assay

The in-house SARS-CoV-2 ELISpot assay was performed by using precoated polyvinylidene difluoride (PVDF) ELISpot^{PRO} 96-wells plates (Mabtech, Nacka Strand, Sweden). The ELISpot procedure was similar to the procedure described previously (Van Gorkom *et al.* 2018), except for the pathogen-specific antigens used to stimulate the PBMCs. For each study participant, eight wells were tested. In the wells, 100 μ l of 2.5×10^6 PBMCs/mL were stimulated with respectively 50 μ L of mitogen control (anti-human CD3 monoclonal antibody (MAb) CD3-2 [0.1 μ g/mL]; Mabtech), and 50 μ L of negative control (AIM-V medium, Life Technologies, Invitrogen, Bleiswijk, The Netherlands).

Wuhan strain-derived spike peptide pool: 50 μ L PepTivator®SARS-CoV-2 Prot_S (a pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the immunodominant sequence domains of the surface (or spike) glycoprotein (“S”) of SARS-Coronavirus 2 (GenBank MN908947.3, Protein QHD43416.1)), 50 μ L of PepTivator®SARS-CoV-2 Prot_S1 (a pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the N-terminal S1 domain of the surface glycoprotein (“S”) of SARS-Coronavirus 2 (GenBank MN908947.3, Protein QHD43416.1)), 50 μ L of PepTivator®SARS-CoV-2 Prot_M (a pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the complete sequence of the membrane glycoprotein (“M”) of SARS-Coronavirus 2 (GenBank MN908947.3, Protein QHD43419.1)), or 50 μ L of PepTivator®SARS-CoV-2 Prot_N (a pool of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the complete sequence of the nucleocapsid phosphoprotein (“N”) of SARS-Coronavirus 2 (GenBank MN908947.3, Protein QHD43423.2)). For these peptide pools we used a minimum of 10 spot-forming units per well as a significant T cell response, based on validation of our in-house ELISpot using a panel of 101 HCWs who were Wantai seronegative (data available upon request).

Delta and Omicron-specific spike peptide pool: We used 50 μ L of a pool of lyophilized peptides that consisted of 80 peptides (Omicron) or 27 peptides (Delta) of the spike protein. Both peptide pools cover only the mutations of the Omicron (B.1.1.529) or Delta (B.1.617.2) variant of SARS-CoV-2 (synthesized by peptides & elephants GmbH, Hennigsdorf, Germany). The final protein concentrations per well have been equalized for comparison. We separately defined the cut-off point for Delta and Omicron-specific peptide pools based on a second panel of HCWs who

were Wantai seronegative, which yielded a cut-off of 4 spots per well (data available upon request).

Serology

The presence of SARS-CoV-2 anti-trimeric spike protein IgG antibodies was measured with the automated LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin Inc, Stillwater, United States), a Chemo Luminescence Immuno Assay (CLIA) system.

The assay detects antibodies against the Trimeric complex, which includes the RBD and N-terminal domain (NTD) sites from the three subunit S1 (the Trimeric complex).

The presence of anti-nucleocapsid protein IgG antibodies was measured with the nucleocapsid-based Mikrogen recomWell SARS-CoV-2 IgG assay (Mikrogen Diagnostik GmbH, Neuried, Germany) on the automated DYNEX DS2®.

Virus neutralisation assay

SARS-CoV-2 neutralization assays were performed as described previously by Rijkers et al. (Rijkers *et al.* 2020) In brief, serum samples were heat-inactivated at 56°C for 30 minutes. Duplicate serial 2-fold dilutions starting at 1:10 were mixed with 100 TCID₅₀ SARS-CoV-2 (strain hCoV-19/Netherlands/ZuidHolland_10004/2020, D614G (WT)) in a 96-wells format and incubated for 1 hour at 35°C and 5% CO₂. Subsequently, VeroE6 cells (1.8 × 10⁴ cells/well) were added and then incubated for 3 days at 35°C and 5% CO₂. The plates were assessed microscopically by scoring 50% protection for cytopathic effect and the titer (VNT₅₀) was defined as the reciprocal value of the serum dilution showing 50% virus neutralization. Titers ≥10 were considered seropositive. All laboratory procedures using live SARS-CoV-2 were performed in a biosafety level 3 facility.

Statistical analysis

Statistical values (ROC curve and p values) were calculated with GraphPad Prism version 9.1.0. Calculation details on the ROC and threshold can be found on:

https://www.graphpad.com/guides/prism/latest/statistics/stat_calculation_details_for_roc_cu.htm. Groups were compared with unpaired one-way ANOVA or Mann–Whitney U test for comparing two or more groups respectively.

Results

Prospectively and longitudinally collected PBMCs and sera from individuals that were part of the ongoing SIR-study were analysed to assess B- and T- cell responses during the course of the vaccination program in the Netherlands. History of exposure to SARS-CoV-2 infection (SARS-CoV-2 convalescent individuals) was based on serostatus as measured by the Wantai Ab ELISA, a semi-quantitative high-validity assay for detection of seroconversion after SARS-CoV-2 infection through detection of total serum antibody binding to the spike receptor binding domain (RBD) of the ancestral B.1.1.7 variant (Geurtsvankessel *et al.* 2020). As expected, baselines IgG levels against SARS-CoV-2 spike (100%) and nucleocapsid (86%) were markedly elevated in SARS-CoV-2 convalescent individuals (figure 1A), indicating a natural humoral immune response (Assis *et al.* 2021). A majority of these convalescent individuals also mounted interferon gamma (INF- γ) T- cell responses against spike, nucleocapsid, and membrane protein-derived peptide pools, indicative of natural cellular immunity (figure 1B). Broad T- cell reactivity, measured as the sum of INF- γ responses to spike, nucleocapsid and membrane antigens, was a good indicator of a

past SARS-CoV-2 infection, with a specificity and sensitivity of 99% and 70% respectively, using a threshold of >10 spot forming cells (figure 1C).

Next, we divided SARS-CoV-2 naive and convalescent individuals into those who were receiving the Pfizer or Janssen vaccine, and tracked their T- and B- cell kinetics over time (figure S1 and table S1). Three weeks after the initial vaccine dose of Pfizer, a marked INF- γ T- cell response was mounted against spike peptides of the ancestral B.1.1.7 variant (figure 3A). The mean response was higher in the convalescent group compared the naive group, although this did not reach significance (48 VS 90 mean SFC respectively). This level of T- cell reactivity was comparable to the response that we observed in hospitalised patients with severe COVID-19, three months after onset of illness (data available upon request). Notably, the second dose of Pfizer, administered three weeks later, did not augment the initial T- cell response (figure 2A). After 8 months, just prior to the booster campaign launched by the Dutch government, vaccine-induced T- cell responses had waned by 2/3 (from ~45 to ~14 mean SFC), regardless of the pre-existing natural immunity based on serostatus. This was re-induced by the subsequent booster vaccine, reaching post-vaccination levels (figure 2A). The kinetics of cellular immunity after the Janssen vector vaccine, comprising of a single dose of the Janssen vector vaccine followed by a booster dose of the Pfizer mRNA vaccine eight months later was largely similar (figure 2B). However, seronegative individuals who received the Janssen vaccine, had significantly lower T- cell responses compared to the other groups (figure 2C). As expected, these spike epitope-based vaccines did not affect the dynamics of cellular immunity to the SARS-CoV-2 nucleocapsid or membrane protein (figure S2).

Next, we separately examined responses to peptides derived from the spike protein of the Delta (B.1.617.2) and Omicron variant (B.1.1.529) after the booster vaccine. Omicron rapidly dispersed globally and superseded the then prevailing Delta variant in the Netherlands at the beginning of 2022, around the time the booster campaign commenced (<https://www.rivm.nl/en/coronavirus-covid-19/virus/variants>). Omicron spike harbours an extensive number of mutations markedly enabling/enhancing the ability of the virus to evade host immunity (McCallum *et al.* 2022). Although the subset of individuals whom we had collected enough cells to perform additional ELISpots was limited in number, we found that presence of pre-existing natural immunity resulted in significantly higher T- cell responses after the booster dose (figure 3). Thus, despite the current vaccines having not yet been updated to incorporate Omicron-specific antigens, spike peptides from the original Wuhan strain still elicit significant cellular immunity against the Omicron variant, supporting the current strategy.

In parallel to cellular immunity, we assessed spike-specific IgG responses and virus neutralisation. As expected, elevated anti-spike IgG at baseline, as measured by a quantitative CLIA assay (Diasorin), was solely detectable in convalescent individuals based on the Wantai assay. Initial dose of a Pfizer or Janssen vaccine induced a higher humoral response in convalescent individuals, beyond the upper detection limit of 2080 BAU/ml (figure 4A and B). The two-dose Pfizer vaccine elicited higher levels than the one-dose Janssen vaccine in immune naive individuals (figure 4E). Importantly, differences in antibody levels corresponded closely to the antibody neutralisation capacity, as measured by SARS-CoV-2 virus neutralisation assays (VNT IC₅₀) (figure 4B and D). At 8 months after vaccination, marked antibody waning

was observed only in naive individuals, which was effectively restored after a booster dose in either vaccine group (figure 4E). Although all participants reached IgG levels that approximated the upper limit of detection after the booster, a combination of natural and vaccine-induced immunity produced higher neutralisation capacity (figure 4E and F).

Taken together, our data suggest that SARS-CoV-2 naive individuals benefit mostly from a booster shot because of significant waning humoral immunity in this group. Cellular immunity after vaccination also waned, but this occurred regardless of the presence of natural immunity. Immune naive individuals that received a Janssen vaccine displayed the lowest level of immunity.

Discussion

Here we present data on T- and B- cell responses after vaccination in healthy volunteers, with and without history of a mild/asymptomatic SARS-CoV-2 infection based on serostatus. Our data indicate that a single dose in convalescent individuals elicits a humoral and cellular immune response that is quantitatively similar compared to a double dose of the Pfizer vaccine in naive individuals. Also, prior natural immunity by and large precludes the waning of antibodies that is seen in immune naive individuals 8 months after vaccination. With regards to cellular immunity, detectable T- cell responses are readily present after initial vaccination, and a second dose of the Pfizer vaccine does not appear to confer augmented T-cell reactivity. T cell responses also decrease over time, regardless of initial serostatus, and a booster dose leads to swift re-induction of these responses. Although naive individuals show prominent re-induction of waning immunity, individuals with pre-existing immunity

from a natural infection harbour the highest responses, including spike-reactive T cells to Omikron variant. This is in accordance with conserved epitopes shared between the Omikron variant and the ancestral strain(Gao *et al.* 2022). Conclusively, our data supports the currently adopted strategy of a single-dose mRNA booster strategy as it strongly re-induces immunity to pre-existing levels, including virus neutralisation. However, despite significant waning we still detect immunity against SARS-CoV-2 at the pre booster phase. Since the pre-defined titre cut-off that provides a meaningful correlate of protection is currently unclear, effectiveness of the booster vaccine cannot be inferred from this data.

Interestingly, when comparing the kinetics of the B- and T- responses, vaccine-induced T- cell immunity wanes across all groups, whereas humoral immunity only wanes in individuals lacking pre-existing natural immunity. Furthermore, a regimen/schedule consisting of 2 doses such as Pfizer, appears to have no additional effect on anti-spike INF- γ T- cell responses, particularly in convalescent individuals. This is in line with recent evidence showing that how infection-primed spike-specific T- cells that are robustly stimulated by an the initial dose of a mRNA vaccine, plateaued in individuals with pre-existing natural immunity(Lozano-Ojalvo *et al.* 2021). Observation of differential kinetics demands for further research into the underpinning mechanisms and the functional impact, in order to determine if pre-existing cellular immunity should be factored into the optimal time interval for future booster vaccinations.

Our study is the first to longitudinally assess in parallel the kinetics of cellular and humoral immunity elicited by a mRNA and vector vaccine in individuals with and

without a history of natural infection with SARS-CoV-2. We intend to extend the follow up on the included health care workers (HCW) to evaluate the persistence of immunity in the long term. The study design has limitations that ought to be mentioned. First of all, a significant proportion of participants could not be sampled across all timepoints due to loss of follow-up. Also, although we excluded HCWs with a history of (re)-infections during the study follow-up in our analysis using self-reported questionnaires, we cannot rule out occurrence of asymptomatic infections. Another limitation was usage of a CLIA-based serological assay with a quantifiable range up to 2080 BAU/ml, which did not allow us to cross-compare IgG levels above this upper limit of detection. Finally, the number of participants with serological evidence of natural immunity receiving the Janssen vaccine was limited in this cohort.

Potential conflicts of interest

All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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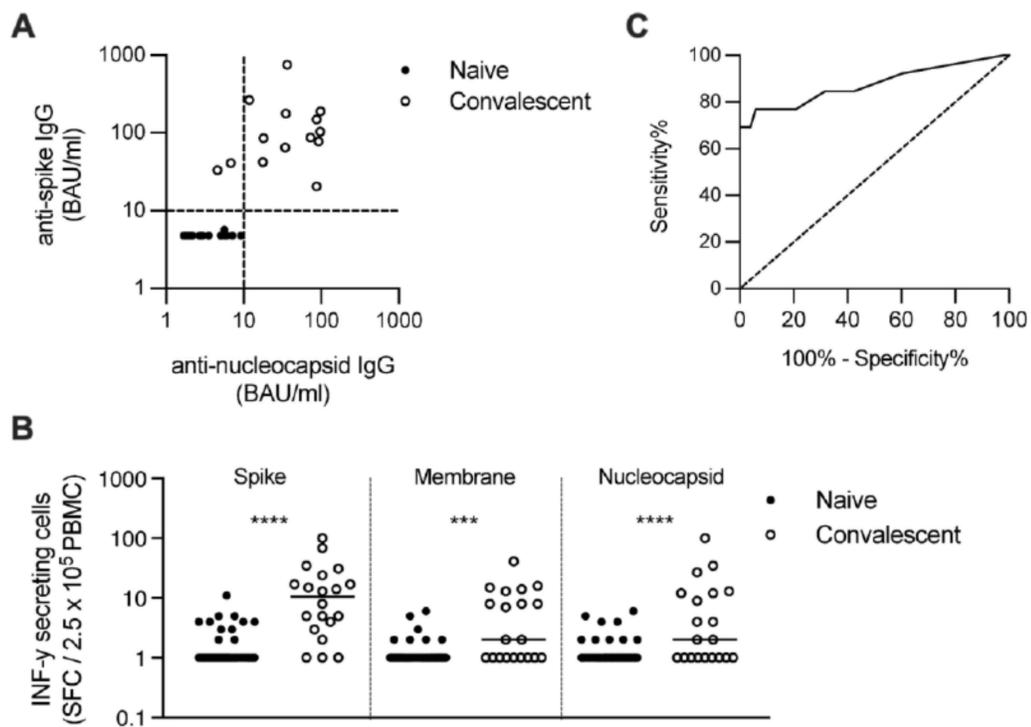


Figure 1. Comparison of T and B cell responses in SARS-CoV-2 naive and convalescent individuals based on serostatus. A) Humoral anti-spike and anti-nucleocapsid responses measured by Wantai assay (in BAU/ml) in SARS-CoV-2 naïve individuals (closed dots) and convalescent individuals (open dots). B) T- cell responses against the SARS-CoV-2 spike, nucleocapsid and membrane peptide pools as measured by INF- γ release ELISpot assay in SARS-CoV-2 naive (closed dots) and convalescent (open dots) individuals. C) Receiver Operating Characteristic (ROC) curve displaying the discrimination performance of the overall T- cell reactivity (SFC reactive to the sum of all peptide pools). *** $P \leq 0.001$, **** $P \leq 0.0001$ (Mann–Whitney U test).

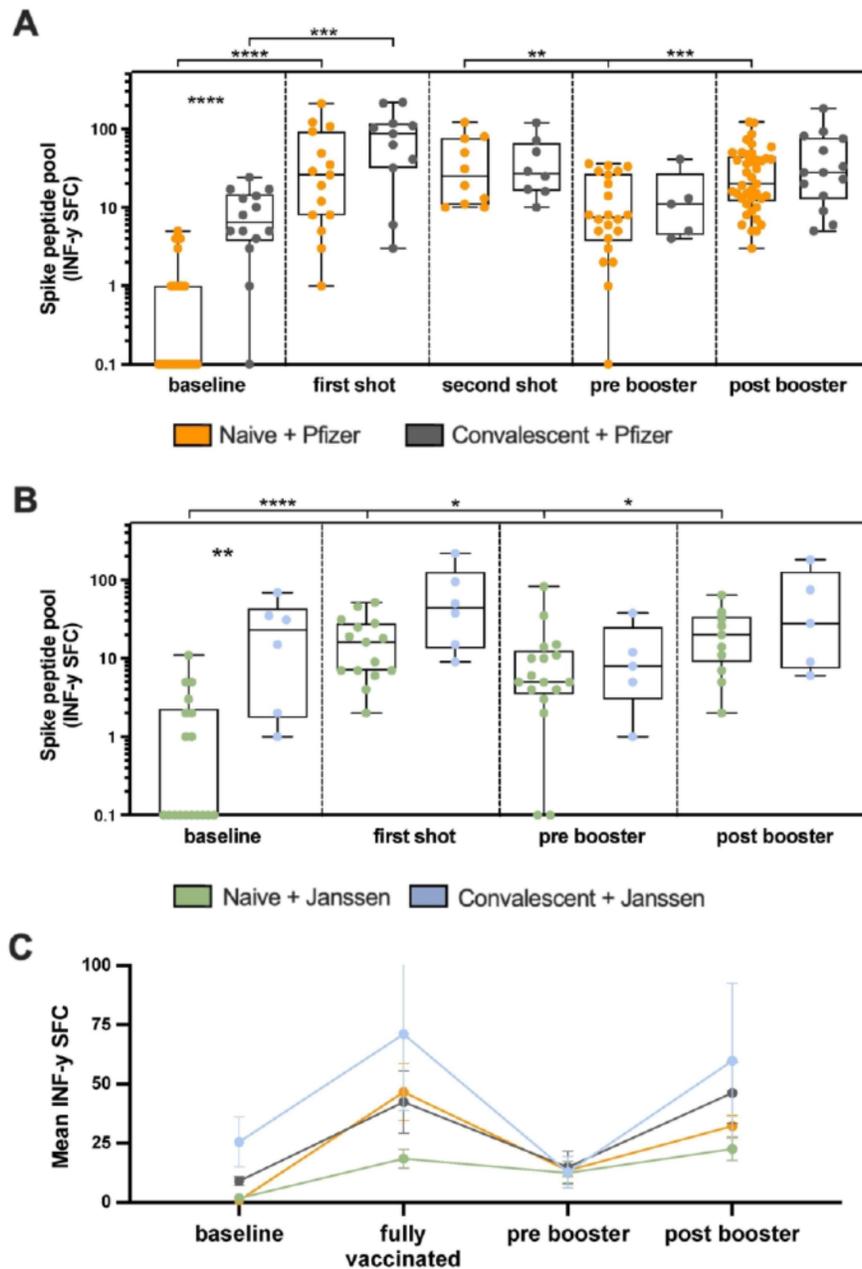


Figure 2. Longitudinal analysis of vaccine-induced INF- γ secreting T-cell responses in individuals with or without a history of exposure to SARS-CoV-2 infection. A) Sum of the mean number of spot-forming INF- γ secreting T-cells (SFC) per 2.5×10^5 PBMCs that reacted to a pool of peptides derived from SARS-CoV-2 spike and specific S1 subunit containing the receptor-binding domain, assessed in individuals with and without evidence of past exposure to SARS-CoV-2 that were

vaccinated with the Pfizer vaccine. B) Similar to A) but in individuals vaccinated with the Janssen vaccine and booster with the Pfizer vaccine. C) Plot of mean \pm s.e.m. T- cell responses (SFC 2.5×10^5 PBMCs) of each group. **P \leq 0.001, ***P \leq 0.001, ****P \leq 0.0001 (Mann–Whitney U tests).

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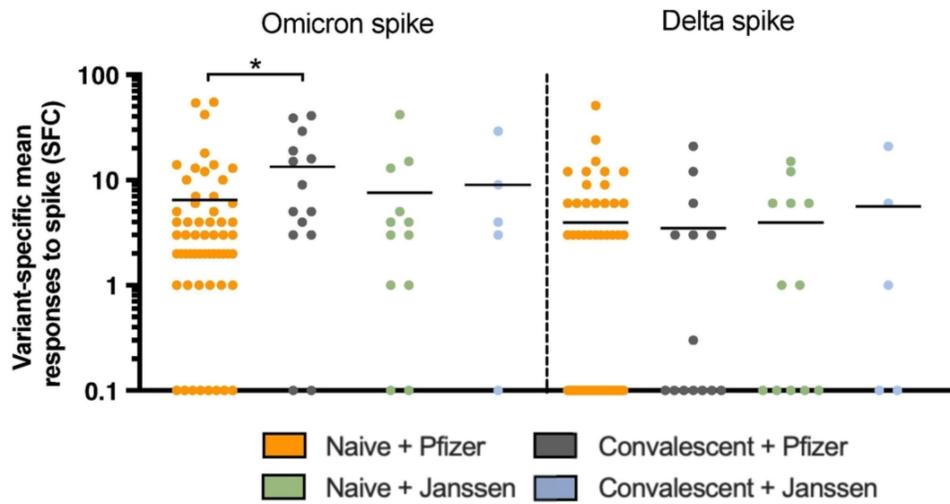


Figure 3. Effect of Pfizer booster vaccine on SARS-CoV-2 Delta and Omicron-specific immune responses against spike. Mean INF- γ secreting T- cell responses in SARS-CoV-2 naive and convalescent individuals against spike peptides from the Omicron variant (left side of graph) and Delta variant (right side of graph).

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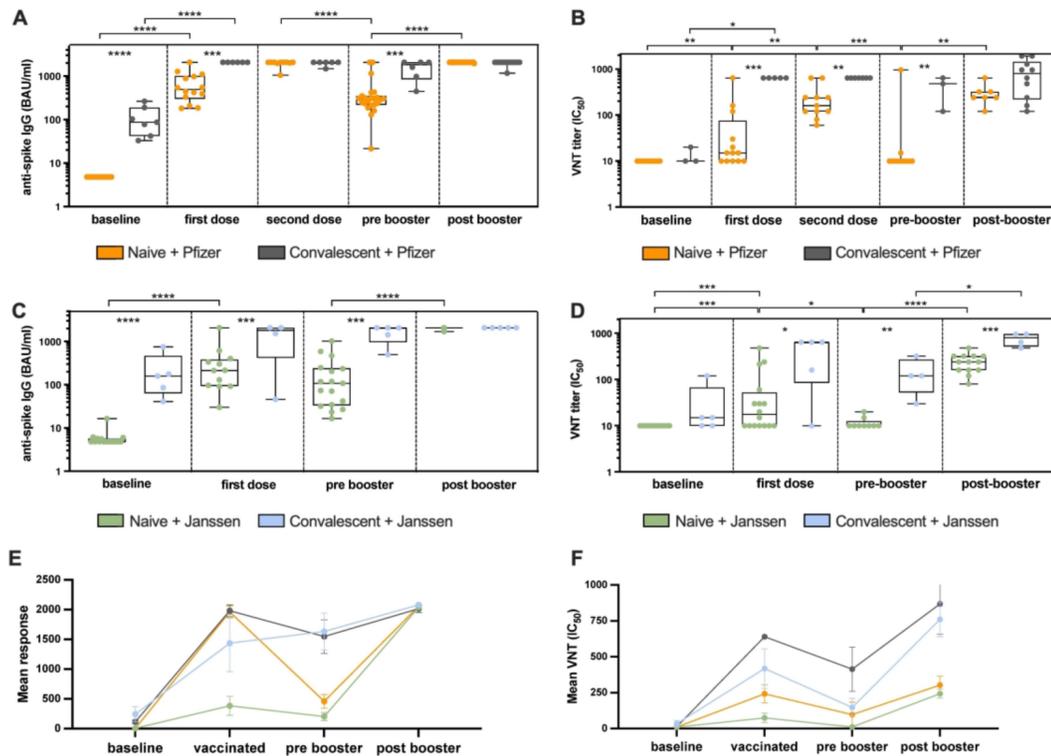


Figure 4. longitudinal analysis of vaccine-induced anti-spike IgG responses in without a history of exposure to SARS-CoV-2 infection. A, C) Anti-spike IgG levels measured by a chemiluminescence immunoassay (CLIA) in naive and convalescent individuals receiving either the Pfizer or Janssen vaccine. B, D) Neutralisation titers measured by SARS-CoV-2 virus neutralisation assays (VNT) in naive and convalescent individuals receiving either the Pfizer or Janssen vaccine. VNT value of 10 means no detectable neutralisation. *** $P \leq 0.001$, **** $P \leq 0.0001$ (Mann–Whitney U tests). E) Mean \pm s.e.m. anti-spike IgG responses in each group. Plotted colored lines match the legends of figure A and B. F) Mean \pm s.e.m. VNT in each group. Plotted colored lines match the legends of figure A and B.