Long-term follow-up of SARS-CoV-2 convalescents reveals distinct magnitude of spike-specific immunity after viral re-exposure and vaccination

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Long-term follow-up of SARS-CoV-2 convalescents reveals distinct magnitude of spike-specific immunity after viral re-exposure and vaccination

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Infection with the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is controlled by the host's immune response¹⁻⁴, but longitudinal follow-up studies of virus-specific immunity to evaluate protection from re-infection are lacking. Here, we report the results from a prospective study that started during the first wave of the COVID-19 pandemic in spring 2020, where we identified 91 convalescents from mild SARS-CoV-2 infection among 4554 health care workers. We followed the dynamics and magnitude of spike-specific immunity in convalescents during the spontaneous course over ≥ 9 months, after SARS-CoV-2 re-exposure and after BNT162b2 mRNA vaccination. Virus-neutralizing antibodies and spike-specific T cell responses with predominance of IL-2-secreting polyfunctional CD4 T cells continuously declined over 9 months, but remained detectable at low levels. After a single vaccination, convalescents simultaneously mounted strong antibody and T cell responses against the SARS-CoV-2 spike proteins. In naïve individuals, a prime vaccination induced preferentially IL-2-secreting CD4 T cells that preceded production of spike-specific virus-neutralizing antibodies after boost vaccination. Response to vaccination, however, was not homogenous. Compared to four individuals among 455 naïve vaccinees (0.9%), we identified 5/82 (6.1%) convalescents with a delayed response to vaccination. These convalescents had originally developed dysfunctional spike-specific immune responses after SARS-CoV-2 infection, and required prime and boost vaccination to develop strong spike-specific immunity. Importantly, during the second wave of the COVID-19 pandemic in fall/winter of 2021 and prior to vaccination we detected a surge of virus-neutralizing antibodies consistent with re-exposure to SARS-CoV-2 in 6 out of 82 convalescents. The selective increase in virus-neutralizing antibodies occurred without systemic re-activation of spike-specific T cell immunity, whereas a single BNT162b2 mRNA vaccination sufficed to induce strong spike-specific antibody and systemic T cell responses in the same individuals. These results support the notion that BNT162b2 mRNA vaccination synchronizes spike-specific immunity in all convalescents of mild SARS-CoV-2 infection and may provide additional protection from re-infection by inducing more rigorous stimulation of spike-specific T cell immunity than re-exposure with SARS-CoV-2.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and rapidly became pandemic⁵⁻⁷. SARS-CoV-2 infections cause a broad range of disease manifestations ranging from mild to severe coronavirus disease 2019 (COVID-19) and have already caused more than 4 million deaths worldwide^{8,9}. SARS-CoV-2 infects cells of the upper

respiratory tract, but can also spread to other cells in the organism due to the widespread distribution of ACE2 that serves as the cellular receptor for viral infection¹⁰. Systemic immune activation with damage to organs and endothelial cells is involved in the transition from mild to severe COVID-19 disease courses¹¹⁻¹³. Early containment of SARS-CoV-2 infection to prevent systemic activation of immunity appears to be important to limit disease manifestation, which is confirmed by the success of COVID-19 vaccination to prevent disease in vaccinees¹⁴⁻¹⁹. SARS-CoV-2 specific immune responses also develop after viral infection²⁰⁻²², but it has remained largely unclear how the immune response of SARS-CoV-2 convalescents reacts to antigen re-encounter either during re-exposure to SARS-CoV-2 or after COVID-19 vaccination²³.

Rapid decline of anti-SARS-CoV-2 IgG and virus-neutralizing antibodies in convalescents

In a prospective study from March 2020 onwards (**Extended Data Figure 1a**), we tested 4554 health care workers of a university hospital for seroconversion to anti-SARS-CoV-2 IgG, and obtained information on the course of infection in each individual (**Extended Data Fig. 1a-c**). Only individuals (91/4554), who had tested positive by PCR for two SARS-CoV-2 genes and/or in whom anti-SARS-CoV-2 IgG against spike antigen or viral nucleocapsid were detected by at least two different serological assays, were considered to have recovered from SARS-CoV-2 infection. All convalescents reported an uncomplicated course of infection, and 20 out of 91 convalescents reported no typical symptoms at all (**Extended Data Fig. 1c**). Anti-SARS-CoV-2 IgG serum levels detected by CLIA in convalescents at month 2 after infection (**Fig. 1a**) decreased significantly in most convalescents three months later (**Fig. 1b,c**). We next determined the virus-neutralization capacity in sera of convalescents using a cell culture SARS-CoV-2 infection inhibition assay. A broad range of virus-neutralization activity was found, with nine convalescents lacking and twelve convalescents showing low virus-neutralization activity (**Fig. 1d**). Virus-neutralization activity also decreased rapidly within three months with the decline being proportional to the initial level of virus-neutralization activity (**Fig. 1e**). The rapid disappearance of spike-specific antibodies may most likely result from rapid control of SARS-CoV-2 infection leading to lack of sustained activation of virus-specific B cells²⁴. Anti-SARS-CoV-2 IgG levels correlated only weakly with virus-neutralization activity in convalescents (**Extended Data Fig. 2a,b**). Of note, virus-neutralization activity but not anti-SARS-CoV-2 IgG levels correlated with reported typical symptoms of SARS-CoV-2 infection in convalescents, but not with sex, age or occupational exposure to COVID-19 patients (**Fig. 1f, Extended Data**

Fig. 2c-f). Our results thus revealed a rapid decline in anti-SARS-CoV-2 IgG and virus-neutralization activity after mild SARS-CoV-2 infection, which confirmed previous reports²⁵, and led us to characterize SARS-CoV-2 spike-specific T cell immunity in convalescents over time.

Figure 1 Dynamics of anti-SARS-CoV-2 IgG levels and virus-neutralization activity after mild SARS-CoV-2 infection.

(a) anti-SARS-CoV-2 IgG levels determined by CLIA from 4554 health care workers (blue - seronegative individuals, red - seropositive individuals) at month 2 after infection. (b) paired analysis of anti-SARS-CoV-2 IgG titers. (c) change (Δ) in anti-SARS-CoV-2 IgG titers at month 5 (y-axis) compared to initial anti-SARS-CoV-2 IgG titers at month 2 (x-axis); LLOD - lower limit of detection. (d) paired analysis of virus-neutralization activity measured as 50% inhibition of viral infection in cell culture (dilution 1:x). 9 convalescents had no detectable and 12 had low neutralization activity. LLOQ - lower limit of quantification; ULOQ - upper limit of quantification. (e) change (Δ) in cell culture neutralization activity (as $\log_2$) at month 5 (y-axis) compared to virus-neutralization activity at month 2 (x-axis), results from 21 individuals were below the quantification limit and are not shown. (f) reporting of symptoms in convalescents and virus-neutralization activity. Median and interquartile ranges are shown, Wilcoxon signed-rank test (b,d), Mann-Whitney test (f); statistical analysis by Spearman correlation and linear regression shown (c,e); r_s denotes Spearman correlation coefficient; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Direct *ex vivo* detection of spike-reactive IL-2-secreting CD4 T cells at high frequencies in convalescents of mild SARS-CoV-2 infection

To determine the frequencies of peripheral blood mononuclear cells (PBMCs) that reacted to the SARS-CoV-2 spike antigen-derived peptides directly *ex vivo*, we used an innovative multiplex Fluorospot-based assay that allows simultaneous detection of IFN γ /IL-2/TNF/GzmB-secretion in single cells. The combination of high sensitivity of ELISPOT technology with multiparametric fluorescence analysis enabled an unparalleled quantitative determination of the frequencies of SARS-CoV-2 spike reactive PBMCs directly *ex vivo* and allowed for evaluation of quantitative intra- and inter-individual changes over time. Using a pool of overlapping peptides (15mers with 11aa overlap) that cover the entire SARS-CoV-2 spike antigen (peptide pool S1 covering aa1-643 and peptide pool S2 covering aa633-1273), we determined the frequencies of spike-reactive cytokine-secreting cells as spot-forming cells (SFCs) per 10⁶ PBMCs. As control, we stimulated PBMCs with a CMV/EBV/Flu (CEF) peptide pool that covers more than 95% of the most frequent caucasian HLA alleles²⁶. This Fluorospot assay had high precision and robustness (**Extended Data Fig. 3a,b**), according to the FDA guidelines for cell-based assays.

Employing Fluorospot analysis for direct *ex vivo* characterization of PBMCs, we found higher frequencies of S1- or S2-reactive mono- and polyfunctional cytokine-secreting cells in convalescents of SARS-CoV-2 infection than in naïve individuals (**Fig. 2a-c, Extended Data Fig. 4a**). Polyfunctional spike-reactive cells constituted approximately 30% of all cytokine-secreting cells (**Fig. 2a**), consistent with induction of functional and protective virus-specific T cell memory, which is characterized by polyfunctionality of T cells²⁷. No increase in frequencies of spike-reactive GzmB-secreting cells was observed in convalescents *ex vivo* (**Fig. 2a,c, Extended Data Fig. 4b**), which pointed towards a low CD8 T cell response. Of note, frequencies of spike-reactive TNF-secreting cells were high in convalescents, but were also elevated in some naïve individuals, especially after S2 stimulation (**Fig. 2a,c, Extended Data Fig. 4c**), indicative of the reported cross-reactivity for S2 peptides with seasonal coronaviruses²⁸. In particular, high frequencies of IL-2-secreting cells (approximately 140 cells per 10⁶ PBMCs) were detected in convalescents (**Fig. 2b,c**). Intracellular staining for cytokines and flow cytometric analysis directly *ex vivo* confirmed the cytokine production by T cells and the dominance of S1- and S2-reactive cytokine-secreting CD4 T cells (**Fig. 2d, Extended Data Fig. 4d**). In contrast, the response rates to CEF peptide stimulation, which is known to be mediated

by CD8 T cells²⁹, were dominated by IFN γ and TNF rather than IL-2-secreting cells in convalescent as well as naïve individuals (**Fig. 2c, Extended Data Fig. 4a-c,e**). Interestingly, CEF-responses were higher in convalescents (**Fig. 2b,c, Extended Data Fig. 4a-c, e**), suggesting that SARS-CoV-2 infection either rendered antigen-specific T cells more responsive to stimulation or had increased their numbers. Thus, the high sensitivity of the Fluorospot assay allowed us to robustly and directly *ex vivo* detect increased frequencies of spike-reactive cells in convalescents after mild SARS-CoV-2 infection with a predominance of IL-2-secreting CD4 T cells.

Notwithstanding the predominant IL-2 profile of spike-reactive cells, we also detected increased frequencies of IL-4- but not IL-5-secreting cells in convalescents (**Fig. 2e**). However, Spearman analysis revealed a direct correlation with anti-SARS-CoV-2 IgG levels and virus-neutralization activity only for S1- or S2-reactive IL-2- and IFN γ -secreting cells, whereas no correlation was found for TNF- or IL-4-secreting cells (**Fig. 2f, Extended Data Fig. 5a,b**). As virus-neutralization activity rapidly declined, the correlation with IL-2 and IFN γ -secreting cells became weaker at later time points (**Extended Data Fig. 5c**), pointing towards a limited value of virus-neutralization activity to indicate cellular anti-viral immunity. Together, these results showed the development of a robust Th1-dominated immune response after mild SARS-CoV-2 infection.

The high inter-assay precision of the Fluorospot assay allowed us to follow virus-specific immunity over time. Compared to the rapid decline of SARS-CoV-2 specific antibodies and virus-neutralization activity, we found a less prominent but still significant decline of the frequencies of spike-reactive IL-2- and IFN γ -secreting cells at month 11 after infection, whereas numbers of TNF-secreting cells remained unchanged (**Fig. 2g,h, Extended Data Figure 5d,e**). However, the proportion of polyfunctional cytokine-secreting cells remained constant (**Fig. 2i**), consistent with sustained and functional virus-specific memory T cell responses after mild SARS-CoV-2 infection.

Figure 2 IL-2 dominated spike-specific T cell responses in convalescents decline over time

(a) *Ex vivo* Fluorospot analysis revealing the frequencies of spike-reactive mono- and polyfunctional cytokine-secreting cells (spot-forming cells/SFCs in 10^6 PBMCs from convalescents (red) or naïve

individuals (blue) at month 5. **(b,c)** total frequencies of spike-reactive cytokine-expressing cells (shown as median $\pm$ SD), CEF (CMV/EBV/Flu) peptide pool as control. **(d)** *ex vivo* detection of the frequencies of CD4 and CD8 T cells of convalescents by intracellular cytokine staining and flow cytometry. **(e)** frequencies of spike-reactive IL-4- or IL-5-secreting cells. **(f)** correlation of frequencies of S1 (upper row) or S2 (lower row)-reactive IFN γ - and IL-2- or IL-4-secreting cells with virus-neutralization activity. **(g-i)** frequencies of spike-reactive cytokine-secreting cells in convalescents at month 5 compared to month 11 after infection. Analyses by ANOVA, Mann-Whitney and Wilcoxon signed-rank tests (b, d, e, g); statistical analysis by Spearman correlation and linear regression (f); r_s denotes Spearman correlation coefficient; n.s. denotes not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Rapid induction of spike-specific immune responses in convalescent and IL-2-secreting cells in naïve individuals after BNT162b2 mRNA vaccination

To evaluate the dynamics of immune responses after vaccination with BNT162b2 mRNA we determined spike-specific T cell and antibody responses in convalescents (n=82) compared to naïve individuals (n=53). Strikingly, in convalescents a rapid augmentation in frequencies of spike-reactive cytokine-secreting mono- and polyfunctional cells was detected already after prime vaccination, which did not further increase after boost vaccination (**Fig. 3a-c, Extended Data Fig. 6a-c**). In contrast, in naïve individuals we detected a more rapid increase in spike-reactive IL-2-secreting cells compared to IFN γ -secreting cells after prime vaccination, which was still lower than in convalescents (**Fig. 3a-c, Extended Data Fig. 6a-c**). After boost vaccination, however, no significant differences were observed anymore for frequencies of spike-reactive cytokine-secreting cells between convalescent and naïve individuals (**Fig. 3a-c, Extended Data Fig. 6a-c**), indicating a synchronization of spike-specific immune responses through vaccination between convalescents and naïve individuals. Moreover, intracellular cytokine staining showed that after prime/boost vaccination mainly CD4 T cells and to a lesser extent CD8 T cells secreted IL-2, IFN γ or TNF in response to S1-/S2-stimulation whereas after CEF-stimulation mainly IFN γ - and TNF-producing CD8 T cells were detected (**Fig. 3d,e, Extended Data Fig. 6d,e**). Of note, frequencies of spike-reactive IL-4-secreting cells were increased after prime/boost vaccination in convalescent and naïve individuals, but were significantly higher in naïve individuals (**Extended Data Fig. 6f**). Taken together, SARS-CoV-2 infection and BNT162b2 mRNA vaccination both preferentially induced IL-2-secreting CD4 T cells. According to a change in national vaccination guidelines during the follow up period for convalescents to receive only a single dose of vaccination, only 33/82 convalescents received both, prime and boost, BNT162b2 mRNA vaccinations.

We continued to analyze the dynamics of spike-specific immune responses by characterizing the increase in virus-neutralization activity after BNT162b2 mRNA vaccination with a competitive CLIA. In convalescents, virus-neutralization activity reached maximal levels already after the prime vaccination (two weeks after prime vaccination 2099 ± 680 ; two weeks after boost vaccination 2022 ± 537 AU/ml), consistent with recent reports^{23,30-32}. In naïve individuals, virus-neutralization activity remained low after the prime vaccination and required a boost vaccination for a further increase (two weeks after prime vaccination 19 ± 110 AU/ml; two weeks after boost 1605 ± 717 AU/ml) (**Fig. 3f**). Since we detected a direct correlation between frequencies of IL-2-secreting cells and virus-neutralization activity in convalescents, it is likely that the early increase of IL-2-secreting CD4 T cells in naïve individuals after BNT162b2 mRNA prime vaccination orchestrated and supported the massive production of virus-neutralizing antibodies after boost vaccination.

Surprisingly, in five SARS-CoV-2 convalescents (6.1%) we observed no increase in virus-neutralizing antibodies after BNT162b2 mRNA prime vaccination (**Fig. 3f, Extended Data Fig. 7a**), indicating a lack of spike-specific immunity after SARS-CoV-2 infection. In comparison, in a separate cohort of 455 naïve individuals receiving BNT162b2 mRNA vaccination only 0.9% (4/455) showed comparably low levels of virus-neutralizing antibodies (**Fig. 3g, Extended Data Fig. 7b**). Boost vaccination in these five convalescents strongly, however, augmented virus-neutralization activity, which then was similar to that observed in naïve individuals after boost vaccination (**Fig. 3g**). Of note, none of these convalescents reported immune suppressive treatment. This suggested that the five convalescents had not generated spike-specific immunity after SARS-CoV-2 infection. Indeed, looking back in the early follow up analyses of these individuals we found a complete lack of virus-neutralization activity as well as low frequencies of spike-reactive cytokine-secreting cells, whereas responses to CEF-stimulation were similar to convalescents who responded to a single vaccination dose with increase in virus-neutralizing antibodies (**Fig. 3h, Extended data Fig. 7c-e**). This excluded a broad downregulation of T cell immunity and indicated a selective lack of spike-specific immunity in these convalescents.

Figure 3 | Spike-reactive IL-2-secreting cells precede development of virus-neutralizing antibodies in naïve individuals as well as convalescents after BNT162b2 mRNA vaccination

(a) frequencies of spike-reactive cytokine-secreting cells in convalescents (red) and naïve individuals (blue) at month 11 after SARS-CoV-2 infection, at 2 weeks after BNT162b2 mRNA prime vaccination

(vacc) and at 2 weeks after boost vaccination (boost). **(b)** heatmap revealing frequencies of individuals bearing S1-reactive mono- or polyfunctional cytokine-secreting cells; c – convalescents, n – naïve individuals **(c)** median and standard deviation for S1-reactive cytokine-secreting cells. **(d,e)** S1-reactive IL-2 and IFN γ -producing CD4 and CD8 T cells determined directly *ex vivo* using intracellular cytokine staining and flow cytometry. **(f)** surrogate virus-neutralization after prime and boost vaccination in convalescents (red), low responder convalescents (grey) and naïve individuals (blue). **(g)** surrogate virus-neutralization in a cohort of 455 naïve individuals (blue), low responders to vaccination (dark grey) and low responder convalescents (light grey) after prime and boost BNT162b2 mRNA vaccination. **(h,i)** frequencies of spike-reactive cytokine-secreting cells at month 5 after infection (h), or month 11, 2 weeks after prime (vacc) and 2 weeks after boost (boost) BNT162b2 mRNA vaccination (i). Statistical analysis by ANOVA, Mann-Whitney and Wilcoxon signed-rank tests (a,e,f,g,h,i). n.s. denotes not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Similar to low antibody responses, four out of five low responder convalescents also showed low increases in the frequencies of spike-reactive cytokine-secreting cells after BNT162b2 mRNA prime vaccination (**Fig. 3i, Extended Data Fig. 7f,g**). After boost vaccination, however, we found increased frequencies of spike-reactive IL-2- but not IFN γ -secreting cells, which then were similar to the frequencies in convalescents and naïve individuals after prime and boost vaccination, respectively (**Fig. 3i, Extended Data Fig. 7f,g**). These results demonstrate that a boost vaccination overcame the low responder state in convalescents with dysfunctional spike-specific immunity. Interestingly, four of the low-responder convalescents did not report any symptoms during SARS-CoV-2 infection, which suggests that low infection doses of SARS-CoV-2 or contact with poorly infectious virus may have caused induction of nucleocapsid-specific antibodies without activating spike-specific T cell responses and generation of virus-neutralizing antibodies.

Distinct dynamics of spike-specific immunity after SARS-CoV-2 re-exposure and BNT162b2 mRNA vaccination

During the second wave of the pandemic in fall and winter 2020/2021, i.e. between month 5 and 11 after the initial infection, and before BNT162b2 mRNA vaccination, we detected in six out of 82 convalescents sharp increases in virus-neutralization activity by more than a factor of 8 (3 log₂) that was confirmed by similar increase of virus-neutralization activity in a competitive and quantitative CLIA (**Fig. 4a,b**). Results from this assay measuring surrogate neutralization activity correlated strongly with cell-culture virus-neutralization activity (**Extended Data Fig. 8a**). No PCR-based diagnostic procedures were performed for detection of SARS-CoV-2 RNA from nasopharyngeal swabs, because none of the individuals reported

symptoms. Surprisingly, in these convalescents no comparably steep rise in nucleocapsid-specific anti-SARS-CoV-2 IgG levels was observed (**Fig. 4c**), indicating a selective surge in virus-neutralizing antibodies after SARS-CoV-2 re-exposure. Strikingly, despite the sharp increase in virus-neutralization activity no increase in frequencies of spike-reactive cytokine-secreting mono- or polyfunctional cells was detected by Fluorospot analysis (**Fig. 4d,e, Extended Data Fig. 8b,c**). This supported the notion that in convalescents SARS-CoV-2 re-exposure was controlled locally in the upper respiratory tract by increased levels of virus-neutralizing antibodies rather than a strong systemic activation of virus-specific T cell immunity.

In sharp contrast, among the naïve individuals (n=53) only one person reported symptoms and a PCR-based diagnostic procedure confirmed SARS-CoV-2 infection (not shown). In this individual, we detected increased virus-neutralization activity and a simultaneous augmentation in anti-SARS-CoV-2 nucleocapsid-specific IgG as well as increased frequencies of spike-reactive IFN γ - and IL-2-secreting cells (**Fig. 4a-c,f, Extended Data Fig. 8d,e**). Together, these data demonstrated a fundamental difference in magnitude of anti-viral immunity to SARS-CoV-2 after infection of a naïve person compared to SARS-CoV-2 re-exposure in convalescents. The large difference in frequencies of individuals with increased virus-neutralizing antibodies after SARS-CoV-2 re-exposure, i.e. 6/82 in convalescents (7.3%) compared to 1/53 (1.9%) in naïve individuals, suggested that virus-specific memory B cells readily responded to a viral challenge. Furthermore, these results indicate that established SARS-CoV-2 specific immunity detected lower numbers of SARS-CoV-2 reaching the upper respiratory tract than are required to establish infection.

After BNT162b2 mRNA prime vaccination, the SARS-CoV-2 re-exposed convalescents showed a swift increase in virus-neutralizing antibodies (**Fig. 4g, Extended Data Fig. 8f**), consistent with the recently reported robust induction of antibodies after mRNA vaccination³³. This suggested a distinct magnitude of spike-specific immunity to viral re-exposure compared to vaccination. Importantly, after vaccination of re-exposed convalescents we further detected increased frequencies of spike-specific IFN γ - and IL-2-secreting cells (**Fig. 4h, Extended Data Fig. 8g,h**). Interestingly, also the previously infected individual showed a further increase in numbers of IFN γ - and IL-2-secreting cells after BNT162b2 mRNA vaccination (**Fig. 4i, Extended Data Fig. 8i,j**). This demonstrated the robust strength of BNT162b2 mRNA vaccination to further boost virus-specific B and T cell immunity in convalescents with recent SARS-CoV-2 re-exposure.

Figure 4 | Selective increase in virus-neutralization activity but not spike-reactive T cells after SARS-CoV-2 re-exposure of convalescents

(a) change (Δ) in cell culture virus-neutralization activity and surrogate virus-neutralization activity in convalescents (red), re-exposed convalescents (6/82) (yellow) (detected in samples obtained at Körber et al. page 13)

months 5, 8 and 11) or in a recently SARS-CoV-2 infected naïve individual (1/53) (turquoise). **(b)** virus-neutralization activities at two time points in the individuals identified in (a); a - denotes values at month 5 or 8, and b - denotes values at month 11 after initial infection. **(c)** change (Δ) in anti-SARS-CoV-2 IgG levels in individuals from (a) using a nucleocapsid-detecting CLIA. **(d)** frequencies of S1/S2-reactive mono- and polyfunctional cytokine-secreting cells in convalescents from (a, yellow) before (month 5, left panel) and after virus re-exposure (month 11, right panel) and convalescents without a surge in virus-neutralization activity (red). **(e)** total frequencies of S1-reactive IFN γ - and IL-2-secreting cells from (d). **(f)** frequencies of cytokine-secreting cells in a recently infected naïve individual from (a, turquoise), uninfected naïve individuals (blue), before (month 5) and after infection (month 11). **(g)** surrogate neutralization activity in convalescents (red) and re-exposed (yellow) before and after BNT162b2 mRNA vaccination. **(h,i)** frequencies of S1-reactive IFN γ - and IL-2-secreting cells before vaccination (month 11), 2 weeks after vaccination (vacc) and 2 weeks after boost vaccination (boost) in convalescents (red) or re-exposed convalescents (yellow), or in naïve individuals (blue) or an infected individual (turquoise). Statistical analysis by Mann-Whitney and Wilcoxon signed-rank tests (e,g,h). n.s. denotes not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Discussion

We identify similarly rapid dynamics but different magnitude of spike-specific immunity in response to virus re-exposure compared to BNT162b2 mRNA vaccination in convalescents of mild SARS-CoV-2 infection. The selective increase in virus-neutralization activity in convalescents after SARS-CoV-2 re-exposure without a systemic activation of spike-specific cytokine-secreting T cells does not exclude a contribution to anti-viral defense of local T cells. Tissue-resident T cells, that have potent effector functions and are involved in efficient local immune surveillance^{34,35}, most likely contributed to efficient anti-viral immunity after viral re-exposure. Such local activation, however, escapes monitoring of immune cells that circulate in peripheral blood³⁶. Our observation, that induction of SARS-CoV-2 specific antibody responses was more frequent in convalescents after viral re-exposure during the second wave of the pandemic compared to newly acquired SARS-CoV-2 infection in naïve individuals, supports the notion that established virus-specific immunity may detect much lower numbers of SARS-CoV-2 in the upper respiratory tract than are required to establish infection. Our results further support the notion that re-exposure to SARS-CoV-2 does not provide a similarly strong stimulus as a BNT162b2 mRNA vaccination. Together with detection of a dysfunctional spike-specific immunity in some convalescents that is corrected by vaccination, our results advocate the use of vaccination in individuals with prior mild SARS-CoV-2 infection to enhance immune protection for prevention of re-infection.

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