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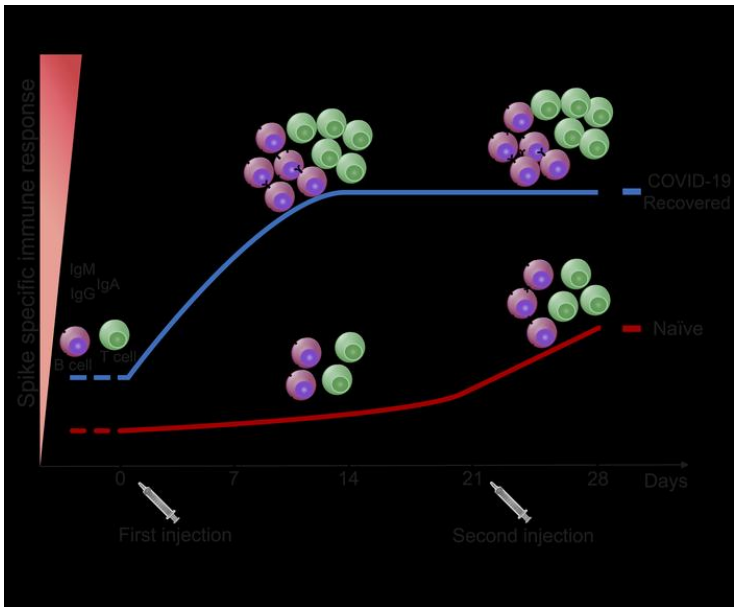
Graphical abstract



First-dose mRNA vaccination is sufficient to reactivate immunological memory to SARS-CoV-2 in recovered COVID-19 subjects

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Abstract

The characterization of the adaptive immune response to COVID-19 vaccination in individuals who recovered from SARS-CoV-2 infection may define current and future clinical practice. To determine the effect of two doses BNT162b2 mRNA COVID-19 vaccination schedule in individuals who recovered from COVID-19 (COVID-19 recovered) compared to naïve subjects, we evaluated SARS-CoV-2 Spike-specific T and B cell responses, as well as specific IgA, IgG, IgM and neutralizing antibodies titers in 22 individuals who received BNT162b2 mRNA COVID-19 vaccine, 11 of which had a previous history of SARS-CoV-2 infection. Evaluations were performed before vaccination and then weekly until 7 days post second injection. Data obtained clearly showed that one vaccine dose is sufficient to increase both cellular and humoral immune response in COVID-19 recovered subjects without any additional improvement after the second dose. On the contrary, the second dose is proved mandatory in naïve ones to further enhance the immune response. These findings were further confirmed at serological level in a larger cohort of naïve (68) and COVID-19 recovered (29) subjects, tested up to 50 days post vaccination. These results question whether a second vaccine injection in COVID-19 recovered subjects is required and indicate that millions of vaccine doses may be redirected to naïve individuals, thus shortening the time to reach herd immunity.

Introduction

As of April 4th 2021, over 130,4 million people have been diagnosed with COVID-19 worldwide, with more than 2,8 million confirmed deaths (1). COVID-19 is associated with high transmission and rising numbers of cases of acute respiratory distress are threatening to overwhelm global health-care capacity (2-5). Up to now, few COVID-19 vaccines based on mRNA or adenovirus have been approved by international and national medicines agencies. For this reason, we are currently facing some difficulties in producing a number of vaccine doses adequate to meet current needs (6-9). Approved COVID-19 mRNA vaccines require a first dose followed 3 to 4 weeks later by a second injection (6-7). In many countries, vaccinations are carried out on all volunteer subjects regardless of past medical history positive for SARS-CoV-2 infection (10). This approach raises two main questions: 1) is it necessary to vaccinate those who recovered from COVID-19? This be the case, 2) is it necessary to administer the second dose of vaccine in this setting?

In this manuscript, we evaluated SARS-CoV-2 Spike-specific T and B cells response, as well as IgG, IgA, IgM and neutralizing antibodies specific titers in 22 individuals, 11 of which had a previous history of COVID-19 (COVID-19 recovered). All recruited subjects were evaluated before receiving BNT162b2 mRNA COVID-19 vaccine and then weekly until 7 days post second injection (administered 21 days after the first dose). Data obtained clearly showed that one vaccine dose is sufficient to increase both cellular and humoral immune response in COVID-19 recovered subjects without any additional improvement after the second dose. These results question whether a second vaccine injection in COVID-19 recovered subjects is indeed required and indicate that millions of vaccine doses may be redirected to naïve individuals.

Results and Discussion

During the first two months of the vaccination campaign against COVID-19 made with BNT162b2 mRNA vaccine in Florence, Tuscany, Italy, we recruited 22 healthcare workers, 11 of which had a previous history of SARS-CoV-2 infection. The demographic and clinical characteristics of the recruited subjects are detailed in Supplementary Table 1. We collected a peripheral blood draw at basal time (time 0, before injection of the first 30 μg dose), then 7 days, 14 days, 21 days (before injection of the second 30 μg dose), and 28 days later (Supplementary Fig.1). The 22 recruited subjects were evaluated at each time point for humoral response and the presence of SARS-CoV-2 Spike-specific T and B cells.

As shown in Figure 1A, anti-nucleoprotein (N) IgG was present before vaccination only in COVID-19 recovered subjects, confirming their previous exposure to SARS-CoV-2 ($p<0.001$). As expected, anti-N IgG levels remained stable over the study period in COVID-19 recovered subjects while undetectable in naïve individuals (Fig.1A). On the other hand, anti-S IgM increased over time in naïve subjects, while exhibiting no substantial changes in COVID-19 recovered individuals (Fig.1B). Pre-vaccination levels of anti-S IgA and IgG were detected only in COVID-19 recovered subjects ($p<0.01$ with Mann-Whitney U test). In naïve individuals, anti-S IgA and IgG increased progressively from 14 to 21 days following the first injection and further grew at day 28, one week after the second dose. On the contrary, in COVID-19 recovered subjects anti-S IgA and IgG massively increased already at day 7 following first injection and remained stable over time (Fig.1C and D). We also measured anti-RBD IgG, whose kinetic appeared similar to that of anti-S IgG in both naïve and COVID-19 recovered individuals (Fig.1E). Interestingly, neutralizing antibodies were already present in the sera of COVID-19 recovered subjects before vaccination but significantly increased after the first BNT162b2 mRNA vaccine dose and did not further grow after the second injection (Figure 1F). On the other side, naïve subjects showed appreciable titers of neutralizing antibodies only one week after the administration of the second vaccine dose and did not reach the levels present in COVID-19 recovered subjects' sera (Figure 1F).

We evaluated by flow cytometry the frequency of B cells capable of recognizing SARS-CoV-2 Spike protein (Figure 2A and Supplementary Figure 2). As shown in Figure 2B, COVID-19 recovered subjects already presented significantly higher frequencies of circulating SARS-CoV-2 Spike-specific B cells before vaccination as compared to naïve individuals ($p<0.001$ with Mann-Whitney U test). In COVID-19 recovered subjects we observed a constant increase of the frequency of Spike-specific B cells up to 21 days, but then it significantly declined after the second injection (Figure 2B). On the contrary, naïve individuals showed an appreciable increase of these cells only one week after the second dose (Figure 2B). Of note, the frequency of Spike-specific B cells at day 28 was significantly lower in naïve than in COVID-19 recovered subjects ($p<0.001$ with Mann-Whitney U test). As shown in Figure 2C, D and E, Spike-specific B cells were predominantly CD27+ IgG+ cells, but CD27+ B cells of the IgM and IgA isotype could also be identified. The kinetics of Spike-specific B cells expressing the three different immunoglobulin classes in COVID-19 recovered subjects was comparable to that described for total B cells (Figure 2C, D, and E). In naïve subjects, the frequencies of Spike-specific CD27+ IgG+, CD27+ IgM+ and CD27+IgA+ cells increased only after the administration of the second vaccine dose (Figure 2C, D, and E).

These observations show that in COVID-19 recovered subjects the first injection of BNT162b2 mRNA vaccine is sufficient to reactivate immunological memory. Moreover, naïve individuals did not exhibit frequency of B cells and serum Ig levels comparable to those presented by COVID-19 recovered subjects, at least at day 28 after the first injection. This was especially true for antibodies with neutralizing capability.

We also studied the Spike-specific CD4+ T cell response, monitoring CD154 surface expression and the production of IL-2, IFN-γ and TNF-α upon in vitro stimulation with peptide pools (Figure 3A and Supplementary Figure 3). Similarly to Spike-specific B cells, circulating Spike-specific CD4+ T cells were present in COVID-19 recovered subjects before vaccination, while were absent in naïve individuals ($p<0.01$ with Mann-Whitney U test) (Figure 3B). Moreover, in COVID-19 recovered subjects we observed a significant increase already at day

7 after the first dose of BNT162b2 mRNA vaccine administration, in contrast to naïve individuals (Figure 3B). This observation was evident not only by evaluating the frequency of CD4+ T cells expressing CD154 and at least one of the three monitored cytokines (IL-2, IFN-γ and TNF-α) (Figure 3B), but also by evaluating the frequency of T CD4+ cells expressing CD154 in association with each individual cytokine (Figure 3 C, D and E). Unlike our findings for Spike-specific B cell response, the frequency of Spike-specific CD4+ T cells showed a reduction in COVID-19 recovered subjects from day 7 to 14, reaching statistical significance only for CD154+IFN-γ+ cells, and did not recover even after the second administration of the vaccine (Figure 3B, C, D and E). On the contrary, as observed for B cells, also T cells showed a significant decrease following the second injection. Naïve subjects instead showed a significant increase in the frequency of Spike-specific CD4+ T cells after the second dose, reaching the frequencies observed in COVID-19 recovered subjects. (Figure 3 B, C, D and E). We then focused on the polyfunctional capability of Spike-specific CD4+ T cells in naïve and in COVID-19 recovered subjects at the end of the vaccination schedule (day 28 after the first injection). As shown in Figure 3F, we observed similar frequencies of Spike-specific CD4+ T cells producing 2 or 3 cytokines in combination. In agreement with these findings, we observed that Spike-specific CD4+ T cells displayed a similar expression pattern of two immune checkpoint molecules, T cell immunoreceptor with Ig and ITIM domains (TIGIT) and programmed cell death protein 1 (PD1) (Figure 3G). Altogether, these data demonstrate that Spike-specific CD4+ T cells, at least those identified basing on CD154 and cytokine expression, at the end of the vaccination schedule have a similar functional potential both in naïve and COVID-19 recovered subjects, thus independently of prior exposure to SARS-CoV-2. This is the first study, to our knowledge, evaluating the early kinetics of cellular immune response following two doses of BNT162b2 mRNA vaccine in naïve and COVID-19 recovered subjects. During the three weeks following the first vaccine injection antibodies, B and T cells specific for SARS-CoV-2 Spike protein progressively increase in naïve subjects. In addition to what has been already demonstrated for antibodies and T cells in preclinical studies (11), also B cells' frequency increases in the circulation after the administration of the second dose.

Neutralizing antibody titers remain low, close to cut-off values, until day 21, but maximally increase post second injection, confirming that the second dose is mandatory in naïve individuals (12). Regarding COVID-19 recovered subjects, our findings suggest that there is a rapid reactivation of both humoral and cellular immunological memory to SARS-CoV-2 Spike protein. Indeed, 7 days after the first injection we observed maximal increase in circulating Spike-specific antibodies, B and T cells. Interestingly, we observed a decrease in the frequency of Spike-specific CD4+ T cells in COVID19 recovered subjects 14 days after the administration of first vaccine dose. We hypothesize that this reduction could be essentially linked to two phenomena: 1) sequestration of specific CD4+ T cells in the lymph node; 2) suppression of specific CD4+ T cells through tolerogenic mechanisms. B cells do not display the same behaviour, given that their frequency progressively increases until day 21. On the contrary, the second injection appears to be ineffective, and it is rather associated to a contraction of both Spike-specific circulating B and T cells in COVID-19 recovered group. Although these data depict the rapid kinetics of vaccine-induced immune response, they were obtained on a relatively small cohort. To further support these findings, we extended the serological evaluations also on a larger cohort of vaccinated healthcare workers. Among 97 total subjects, 29 had a history of prior SARS-CoV-2 infection, dated 6-9 months before vaccination. Demographic and clinical data of this extended cohort are included in Supplementary Table 2. Subjects were tested before BNT162b2 mRNA vaccination, at day 21 (before the second injection) and at day 50. As shown in Figure 4, anti-Spike IgG, anti-RBD IgG and neutralizing Ab in naïve individuals increased at day 21 and further grew at day 50. COVID-19 recovered subjects showed higher anti-Spike IgG ($p<0.001$ with Mann-Whitney U test), anti-RBD IgG ($p<0.001$ with Mann-Whitney U test) and neutralizing Ab ($p<0.001$ with Mann-Whitney U test) than naïve individuals, before vaccination. Anti-Spike and anti-RBD IgG levels maximized at day 21 and remained stable after the second injection until day 50 (Fig. 4A-B). Interestingly, neutralizing Ab peaked at day 21, but then significantly declined at day 50 (Fig. 4C). Collectively, these data confirm what emerged from the study of vaccine-induced immunity in the smaller cohort. Our observations are in agreement with those recently

published (13-14) and those presented in the accompanying article by the group of Maria Rescigno, confirming that in COVID-19 recovered subjects one dose of BNT162b2 is sufficient to maximize Spike-specific antibody titers. This information has practical fundamental implications. In most countries, individuals who recovered from SARS-CoV-2 infection are not excluded from COVID-19 vaccination, and they commonly follow a two-doses vaccination schedule (15-16). However, differently from naïve individuals, our data suggest that one single injection may be sufficient to protect COVID-19 recovered subjects, in accordance with emerging data from other groups (17-18). This is further supported by the results of clinical trials showing that the immunization levels induced by vaccination in naïve subjects are sufficient to confer protection in 95% of the subjects (6).We also cannot exclude that the second injection might even be detrimental in this context, possibly leading to a functional exhaustion of Spike-specific lymphocytes (19). Indeed, we observed a decrease in the frequency of both B and T cells at day 28 (one week after second dose), but also a decline in the titer of neutralizing Ab at day 50. This is an intriguing hypothesis, but further studies are needed to fully prove it. Our data demonstrate that circulating Spike-specific CD4+ T cells have a similar polyfunctional capability and immune checkpoint expression pattern in naïve versus COVID-19 recovered subjects. However, we cannot exclude the presence of exhausted Spike-specific CD4+ T cells that cannot be reactivated ex vivo. It should be noted that our COVID-19 recovered cohort includes subjects with a history of symptomatic COVID-19 infection. It is known that the strength of the immune response to SARS-CoV-2 directly correlates to disease severity both in the acute phase (20-21) and in the memory phase (22) and that memory immune response to SARS-CoV-2 is quite heterogeneous in the months after recovery (23). For this reason, additional studies are required to understand whether one single mRNA vaccine administration may be sufficient also in people with a history of asymptomatic SARS-CoV-2 infection or in those presenting low levels of both humoral and cell mediated antigen-specific immune response. Additional information is also urgently needed on other approved COVID-19 vaccines. It should also be noted that our data have been obtained on a cohort of subjects who experienced SARS-CoV-2 infection 6-9 months

before vaccination. Therefore, we do not know if a single vaccine injection might be equally effective in people who recovered from infection since longer periods. Additional time is clearly needed to answer this question, given the recent outbreak of COVID-19.

Although approved by international and national medicines agencies, the distribution and administration of vaccines is still limited to a minority of the global population. Saving one vaccine dose for each person who recovered from COVID-19 would substantially increase the number of doses available for naïve individuals, thus shortening the time to reach herd immunity.

Methods

Patients

Demographic and clinical information about the enrolled subjects is available in Supplementary Methods.

Flow cytometry

Full protocols for the identification of Spike-specific T CD4+ and B cells are available in Supplementary Methods.

Detection of SARS-CoV-2 specific Ig

SARS-CoV-2 specific Ig and neutralizing Ab were quantitated following manufacturer's instructions, as detailed in Supplementary Methods.

Study approval

The procedures followed in the study were approved by the Careggi University Hospital Ethical Committee. Written informed consent was obtained from recruited patients.

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Author Contributions

A.M., N.D.L, F.L., G.M.R., L.C., A.B., F.A., designed the study; N.D.L., E.M., M.S., L.Z. F.L. collected peripheral blood samples and collected informed consent; A.M., L.M., L.S., A.V., M.C., G.L., S.T.K., M.G.C., A.R. performed experiments; P.P., C.S, L.T., provided advice; A.M., L.M., analyzed data; A.M., L.S., F.A. wrote the manuscript. All authors revised the manuscript and gave final approval.

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Figures

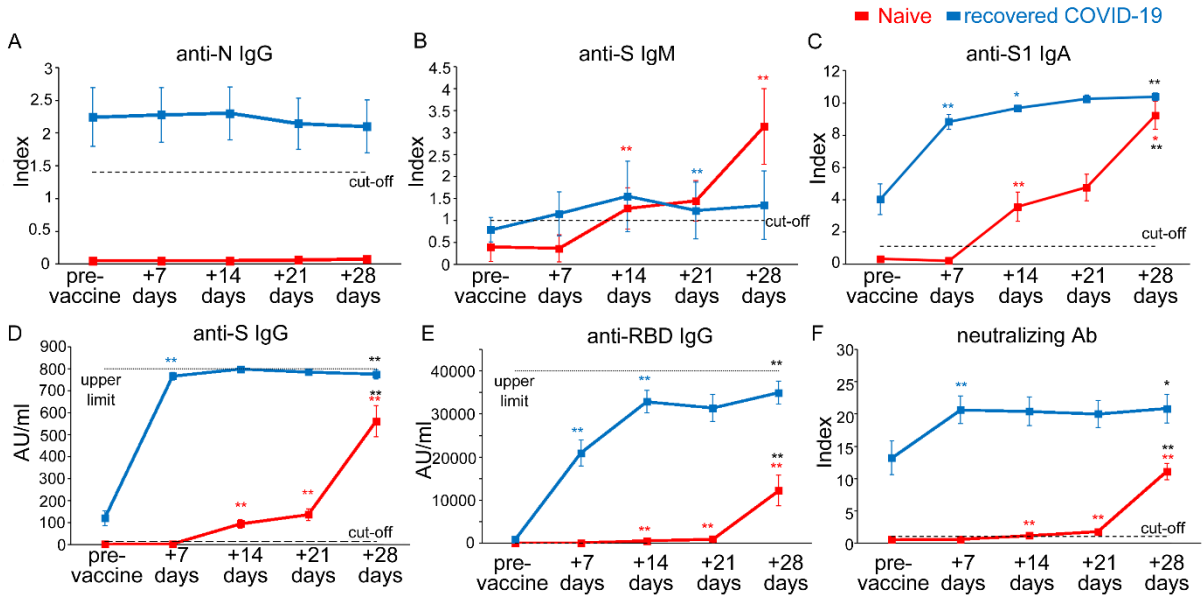

Figure 1. Evaluation of anti-SARS-CoV-2 serum antibody levels

Evaluation of anti-N IgG (A), anti-S IgM (B), anti-S IgA (C), anti-S IgG (D), anti-RBD IgG (E), S-neutralizing antibodies (F) in naïve (red lines) and COVID-19 recovered (blue lines) subjects before and after 7, 14, 21, 28 days of first vaccine administration. Data are represented as mean ±SE from 11 naïve and 11 COVID-19 recovered subjects (A,B,D) or from 6 naïve and 6 COVID-19 recovered subjects (C, E). Dashed lines represent cut-off values, dotted lines are upper detection limits. Blue and red asterisks refer to paired statistics within each study group compared to the previous time point in the kinetic. Black asterisks at day 28 represent paired statistic compared to pre-vaccine point. *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$ calculated with Wilcoxon-Signed Rank test.

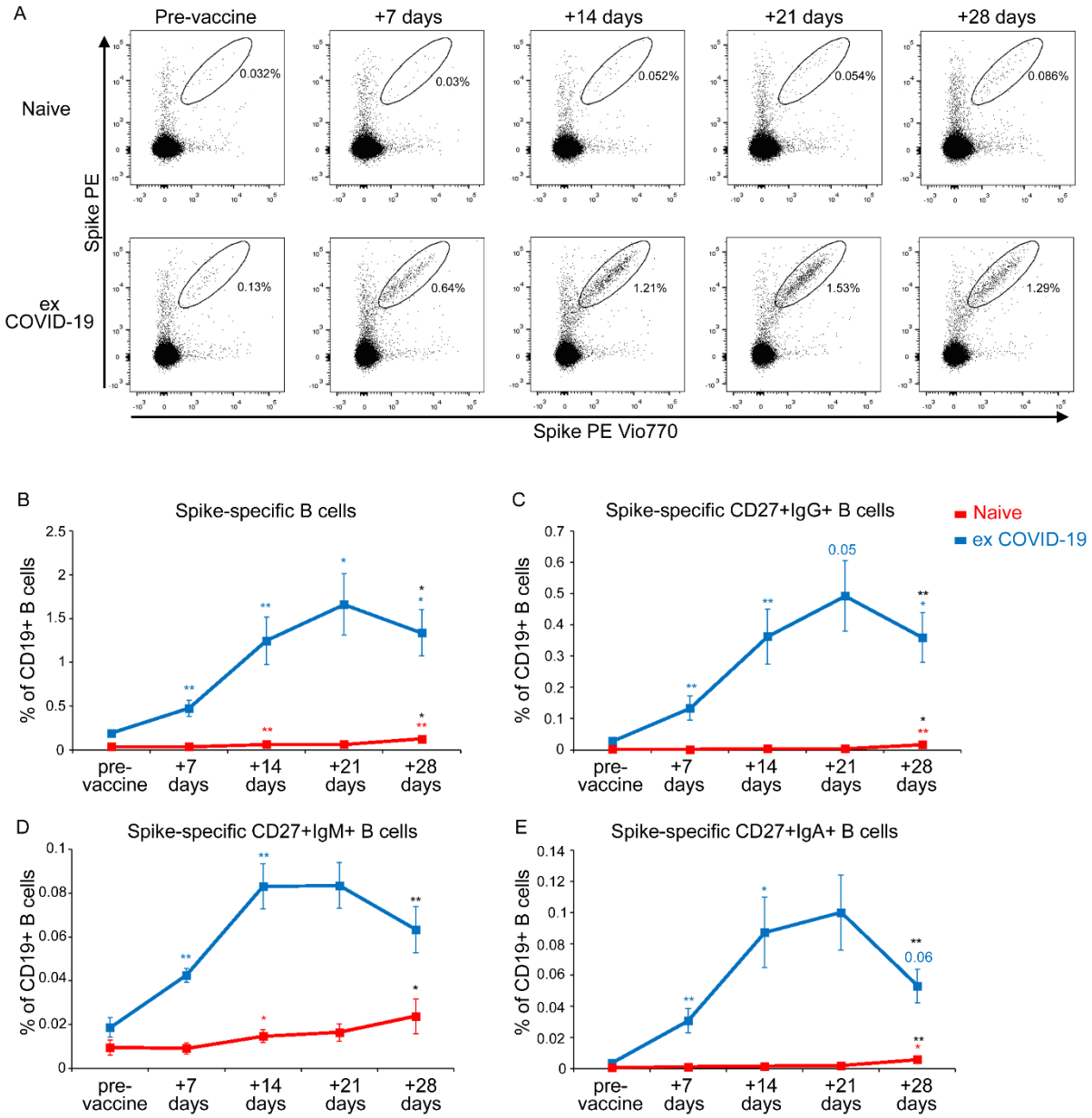

Figure 2. Detection of Spike-specific circulating B cells in naïve and COVID-19 recovered vaccinated subjects

(A) Representative flow cytometric plots of Spike-specific B cells in one naïve (upper row) and one COVID-19 recovered subject (lower row) before vaccination and after 7, 14, 21, 28 days from the first injection. Kinetic analysis of frequencies of total (B), CD27+ IgG+ (C), CD27+ IgM+ (D), CD27+ IgA+ (E) Spike-specific B cells in naïve (red lines) and COVID-19 recovered (blue lines) subjects, before and after vaccination. Data are represented as mean ±SE from 11 naïve and 11 COVID-19 recovered subjects. Blue and red asterisks refer to paired statistics within each study group compared to the previous time point in the kinetic. Black asterisks at day 28 represent paired statistic compared to pre-vaccine point. *= p<0.05; **= p<0.01; ***= p<0.001 calculated with Wilcoxon-Signed Rank test. .

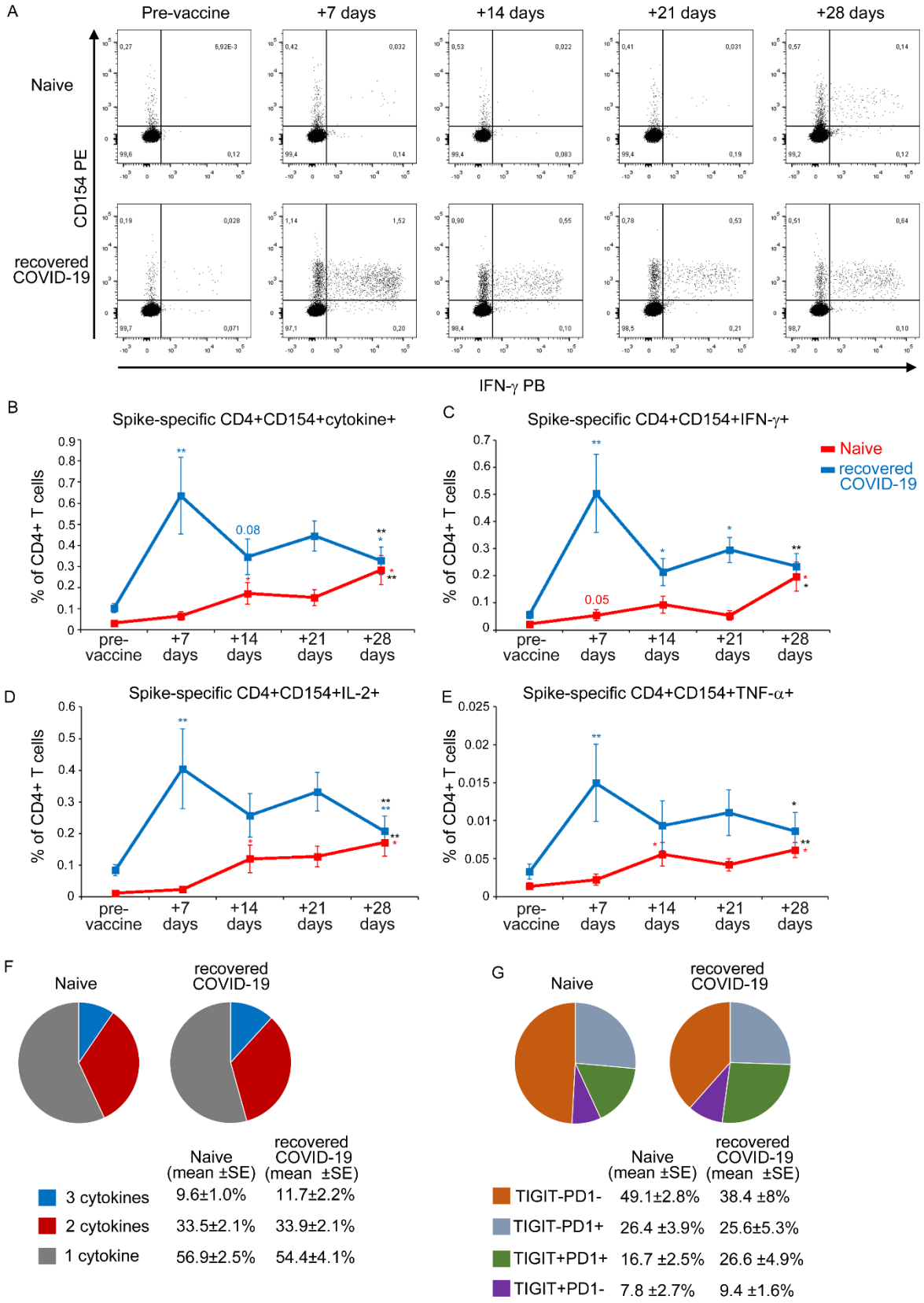

Figure 3. Detection of Spike-specific circulating CD4+ T cells in naïve and COVID-19 recovered vaccinated subjects

A) Representative flow cytometric plots of Spike-specific CD4+ CD154+ IFN-γ+ T cells in one naïve (upper row) and one COVID-19 recovered (lower row) subject before vaccination and

after 7, 14, 21, 28 days from the first injection. Kinetic analysis of frequencies of CD154+ cells producing at least one cytokine among IL-2, IFN-γ and TNF-α (B), CD154+ IFN-γ+ (C), CD154+ IL-2+ (D), CD154+ TNF-α+ (E) Spike-specific T cells in naïve (red lines) and COVID-19 recovered (blue lines) subjects, before and after vaccination. Data are represented as mean ±SE from 11 naïve and 11 COVID-19 recovered subjects, subtracted of background unstimulated negative control. Blue and red asterisks refer to paired statistics within each study group compared to the previous time point in the kinetic. Black asterisks at day 28 represent paired statistic compared to pre-vaccine point. *= $p<0.05$; **= $p<0.01$; ***= $p<0.001$ calculated with Wilcoxon-Signed Rank test. (F) Characterization of SARS-CoV-2 Spike-specific CD4+ T cell polyfunctionality in naïve and COVID-19 recovered subjects at day 28 following the first vaccine injection. Results are expressed as mean percentages from 11 naïve and 11 COVID-19 recovered subjects. (G) Characterization of TIGIT and PD1 expression by SARS-CoV-2 Spike-specific CD4+ T cells in 7 naïve and 8 COVID-19 recovered subjects. Results are expressed as mean percentages.

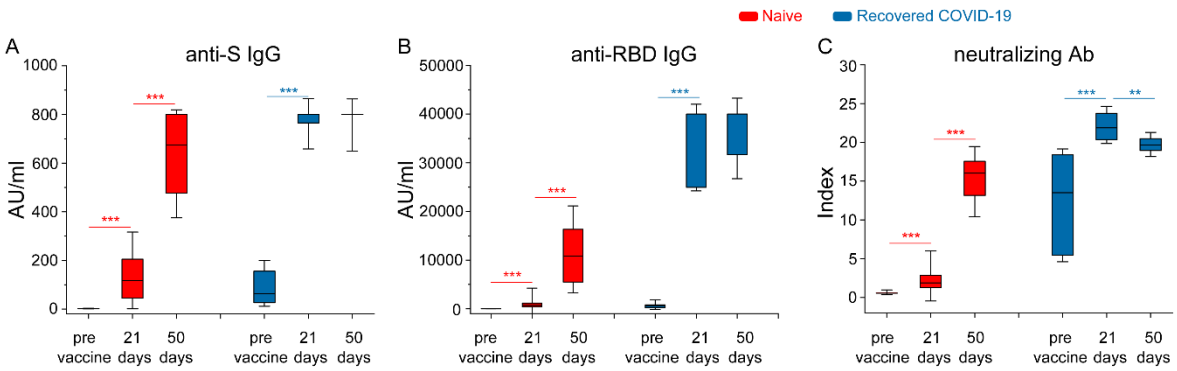

Figure 4. Characterization of vaccine-induced anti-Spike humoral response up to 50 days post vaccination

Evaluation of anti-S IgG (A), anti-RBD IgG (B), S-neutralizing antibodies (C) in naïve (red box) and COVID-19 recovered (blue box) subjects before and after 21 and 50 days of first vaccine dose administration. Box-plot represent 25th-75th percentiles. Black line represent the median. Whiskers represent SE. Data have been obtained from 68 naïve and 29 COVID-19 recovered subjects. **= p<0.01; ***= p<0.001 calculated with Wilcoxon-Signed Rank test.