

Immune Response to SARS-CoV-2 Vaccine in 2 Men

Sudhir Gupta Houfen Su Sudhanshu Agrawal

Division of Basic and Clinical Immunology, University of California Irvine, Irvine, CA, USA

Keywords

COVID-19 · Vaccine · Memory T cells · Memory B cells · Regulatory lymphocytes · Follicular helper T cells · SARS-CoV-2-specific CD8 T cells

Abstract

Introduction: In the trials of corona virus vaccines, detailed analyses of subsets of lymphocytes were not carried out. We present perhaps the most comprehensive immunological analysis of 29 subsets of B and T cells in 2 healthy subjects receiving 2 doses of the Pfizer SARS-CoV-2 (COVID-19) vaccine. **Methods:** Analyses were performed prior to vaccination, 3 weeks following the 1st dose, and 4 weeks following the 2nd dose. Total, naïve (T_N), and different memory and effector subsets (T_{CM} , T_{EM} , and T_{EMRA}) of CD4+ and CD8+ T cells; SARS-CoV-2 spike protein-specific tetramer+, and cytotoxic CD8+ T; subsets of T follicular cells (T_{FH} , T_{FH1} , T_{FH2} , $T_{FH1/17}$, and T_{FH17}); B-cell subsets (mature B cells, naïve B cells, transitional B cells, marginal zone B cells, class-switched memory B cells, germinal center B cells, and CD21^{low} B cells), and plasmablasts; and regulatory lymphocytes (CD4+ Treg, CD8+ Treg, Breg, and T_{FR} cells) were evaluated with specific monoclonal antibodies by flow cytometry. **Results:** A lack of COVID-19 IgG antibodies after the 1st dose in one of 2 subjects was associated with increased regulatory lymphocytes

and decreased plasmablasts. Seroconversion after the 2nd dose in this subject was associated with decreased T_{FR} cells and increased plasmablasts. In both subjects, CD4 T_{EM} and CD8 T_{CM} were markedly increased following the 2nd dose. T_{FH1} and regulatory lymphocytes were increased (except Breg) following the 1st dose. A striking increase in SARS-CoV-2-specific CD8+ T cells was observed following the 2nd dose.

Conclusion: Our data support the need for 2nd dose of vaccine to induce strong SARS-CoV-2 CD8 T-cell specific response and generation of memory subsets of CD4+ and CD8+ T cells. Regulatory lymphocytes appear to play a role in the magnitude of response.

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Introduction

The COVID-19 pandemic has to date caused >217 million infections and 4.51 million deaths worldwide, and 39.2 million infections and 639,000 deaths in the USA. In December 2020, 2 mRNA vaccines (Pfizer and Moderna) were given an Emergency Use Authorization by the Food and Drug Administration. Pfizer vaccine has recently been given full use authorization. In March 2021,

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Johnson & Johnson vaccine has also received the Emergency Use Authorization. Both mRNA vaccines are given in 2 doses, 3–4 weeks apart. They are reported to provide 94–95% protection from transmission of the virus. In comparison, the Johnson & Johnson vaccine is a single-dose vaccine with approximately 70% protection against transmission of SARS-CoV-2 infection. During clinical trials of these vaccines, a major emphasis was placed on the production of neutralizing antibodies, and very little emphasis was given to T-cell responses [1–3].

Clinical manifestations of patients infected with SARS-CoV-2 virus range from asymptomatic to severe COVID-19 clinical symptoms and even death. The development of both antibody and T-cell responses has been reported [4–14]. More recently, Dan et al. [15] reported the presence of both T- and B-cell memory, and neutralizing antibodies up to 6–8 months post-infection in convalescent individuals.

Neutralizing antibodies play an important role in the prevention of viral infections; however, their role in determining the course of disease is unclear. By contrast, cytotoxic CD8+ T cells (CTLs) play an important role in the clearance of viruses. Therefore, these CTLs may influence the clinical outcome of mild versus serious disease.

In this study, we present comprehensive analysis of various subsets of CD4+ T cells and CD8+ T cells, including memory and effector CTLs, SARS-CoV-2-specific CD8 T cells, subsets of B cells including class-switched memory (CSM) B cells, germinal center (GC) B cells, and antibody secreting cells (plasmablasts), as well as various members of regulatory lymphocyte clubs in 2 healthy individuals prior to and following the 1st and the 2nd vaccination. Our data demonstrate increased immune responses following the 2nd vaccination, especially T_{CM} and T_{EM} subsets. Alterations in CD4 Treg, CD8 Treg, and T_{FR} may play a role in the generation of effective T and antibody responses.

Materials and Methods

Subjects

A 76-year-old healthy male (subject 1) and a 52-year-old male (subject 2) received both doses of Pfizer COVID-19 vaccine 3 weeks apart. Vaccination was uneventful. Blood samples were drawn 2 weeks prior to the 1st vaccination, 3 weeks after the 1st vaccination, and 4 weeks after the 2nd vaccination. The Institutional Review Board (Human) of the University of California at Irvine approved the protocol. Written consent that allows publication of data was obtained from the subject. The subjects have no known history of any illnesses, including hypertension, diabetes, kidney disease, psychological illness, or family history of genetic

diseases. Subjects are HLA-A*0201+ and have served as healthy control donors for our laboratories for >3 years and donating blood every 4–6 months. Their lymphocyte subsets have been stable with minimal fluctuations of 5–7%. Patients tolerated both doses without any side effects.

Antibodies and Reagents

The following monoclonal antibodies and their isotype controls were purchased from various sources. Biolegend (San Diego, CA, USA): CXCR5 AL488, CCR6 PE, CD38 BV 650, CD45RO BV421, CD127 BV510, CD4 BV650, CD24 BV510, HLA-DR Percp, CCR7 BV510, CD45RA BV650, CD8a AL700, CCR6 BV650, CD45RA BV510, CXCR3 BV421, CD4 Percp, CD8 Percp, CD8a BV605, CD19 Percp; BD Biosciences (San Jose, CA, USA): CD25 APC, FoxP3 PE, Mouse IgG1PE, CD25 FITC, ICOS AL647, PD-1 PE, CD27 FITC, IgD PE, IgM APC, CD21 BV421, CD107a PE, GranzymeB AL647, Perforin FITC; MLB International (Woburn, MA, USA): HLA-A*02:01 SARS-CoV-2 Spike Glycoprotein Tetramer YLQPRTFL.

Flow Cytometry

Phenotypic analysis was performed on whole blood preparations. 200 μ L blood was used per combination for antibody staining. Following lysis using lysing buffer (BD Pharmaceuticals, San Jose, CA, USA), cells were washed and fixed by 2% PFA, and stained with monoclonal antibodies and isotype controls for 30 min at room temperature.

Regulatory Lymphocytes

Cells after surface staining were fixed and permeabilized by Foxp3 staining buffer set (BD Bioscience) as per manufactures' protocol, and were incubated with Foxp3 PE monoclonal antibody and appropriate isotype control (mouse IgG1k-PE).

SARS-CoV-2-Specific Tetramer Staining

200 μ L blood was mixed with 5 μ L CD8PerCP monoclonal antibody and 10 μ L HLA-A*0201 spike Tetramer PE, vortexed gently, and incubated for 30 min at room temperature protected from light. Red blood cells were lysed using 1 mL of Lyse Reagent supplemented with 0.2% formaldehyde Fixative Reagent per tube. Tubes were centrifuged at 150 g for 5 min, and supernatants were removed. Three milliliters of FACS buffer was added, and the tubes were centrifuged at 150 g for 5 min. Cell pellets were resuspended in 500 μ L of phosphate buffer saline and 0.1% formaldehyde, and stored at 4°C for 1 h in the dark prior to analysis by flow cytometry.

Cytotoxic CD8+ T Cells

Cells were incubated for 30 min with CD8PerCP and CD107a PE (a degranulation marker) for surface staining, lysed, fixed and permeabilized by Fix Perm buffer (BD Biosciences), and then incubated with Granzyme B AL647 and Perforin FITC monoclonal antibodies and appropriate isotype control.

All Fluorescence Minus One controls and isotype controls were stained and fixed by 2% PFA for flow cytometry. Cells were acquired by BD FACS Celesta (Becton-Dickenson) and equipped with BVR laser. Forward and side scatters and singlets were used to gate and exclude cellular debris. 30,000 cells were acquired and analyzed using FlowJo software (Ashland, OR, USA).

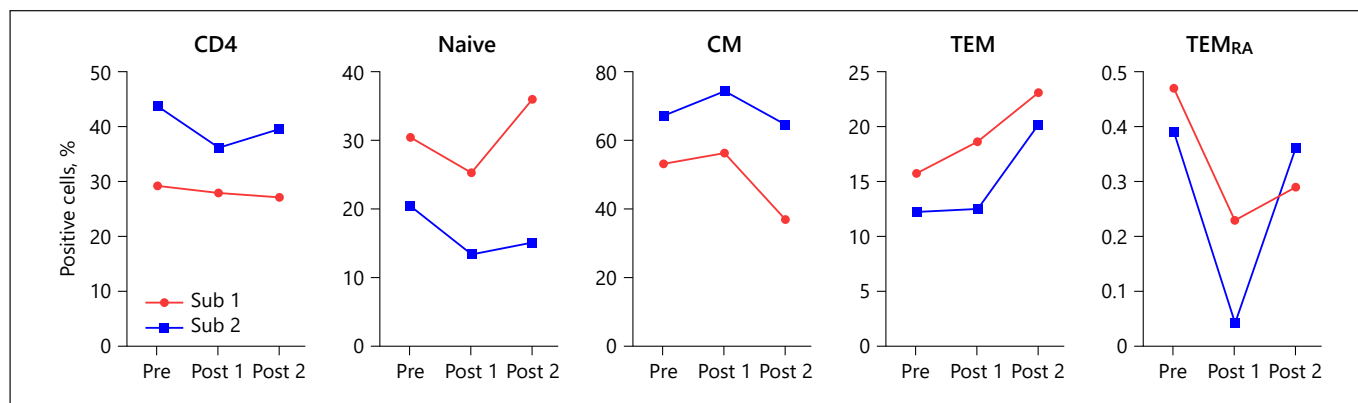


Fig. 1. Effect of vaccine on subsets of CD4+ T cells: T_N and T_{EMRA} were decreased following 1st vaccination followed by partial recovery of T_{EMRA} after the 2nd dose of vaccine. T_{EM} cells were markedly increased after the 2nd dose. T_{EM} , effector memory T cells; T_{EMRA} terminally differentiated effector memory T cells; T_N , naïve T cells.

Markers Used for Various Subsets

B-cell subsets were identified by the following cell surface markers: naïve B cells: CD19+CD27-IgD+IgM+, transitional B cells: CD19+CD38+IgM++, marginal zone (MZ) B cells: CD19+CD27+IgD+IgM+, IgM memory B cells: CD19+CD27+IgM+, CSM B cells: CD19+CD27+IgD-IgM-, GC B cells: CD19+IgD-CD27+/CD38+, plasmablasts: CD19+CD38++IgM-, mature B cells: CD21^{high}CD19+CD38-, CD21^{low} B cells: CD19+CD38-CD21^{low}, Breg: CD19+CD24+CD38+.

Subsets of CD4+ and CD8+ T cells were identified with the following cell surface markers: naïve (T_N): CD4+/CD8+CD45RA+CCR7+, central memory (T_{CM}): CD4+/CD8+CD45RA-CCR7+, effector memory (T_{EM}): CD4+/CD8+CD45RA-CCR7-, and terminally differentiated effector memory (T_{EMRA}): CD4+/CD8+CD45RA+CCR7-, CD8 Treg: CD8+CD183+ICOS+CD25^{high}FoxP3+, CD4+Treg: CD4+CD25^{high}-CD127-FoxP3+.

Subsets of follicular helper T (T_{FH}) cells were identified with the following surface markers: cT_{FH} : CD4+CXCR5+CD45RA-, T_{FH1} : CD4+CXCR5+CD45RA-CCR6-CXCR3+, T_{FH2} : CD4+CXCR5+CD45RA-CCR6-CXCR3-, T_{FH17} : CD4+CXCR5+CD45RA-CXCR3-CCR6+, $T_{FH1} + T_{FH17}$: CD4+CXCR5+CD45RA-CCR6+CXCR3+, T_{FR} : CD4+CXCR5+CD45RA-CD25^{high}FoxP3+.

Results

Effect of Vaccine on Subsets of CD4+ and CD8+ T Cells

T_N cells following antigenic stimulation undergo proliferation and differentiation into effector and memory populations. Based on their migration properties, expression of chemokine receptors, and distinct functions, they have been divided into T_{CM} , T_{EM} , and T_{EMRA} cells [16–19]. We have analyzed these subpopulations of CD4+ and CD8+ T cells prior to and following vaccination.

Effect of Vaccine on CD4+ T Cells and Its Subsets

Data are shown in Figure 1. T_N cells and T_{EMRA} were decreased following the 1st dose, whereas a modest increase in T_{CM} was observed. Following the 2nd dose, a marked increase in T_{EM} cells and a partial recovery in T_{EMRA} cells was observed.

Effect of Vaccine on CD8+ T Cells and Subsets

A marked decrease in CD8+ T cells was observed following the 1st dose with little or no recovery following the 2nd dose. T_{CM} cells were unchanged following the 1st dose; however, following the 2nd dose, a marked increase was observed. Changes in T_{EM} and T_{EMRA} following the 1st and the 2nd dose were different among 2 subjects (Fig. 2).

We also analyzed de novo SARS-CoV-2-specific CD8+ T cells, using specific tetramers and functional CTLs as determined by the expression of degranulation marker CD107a, along with perforin (Fig. 3). After the 2nd dose, an increase in functional (CD107a+) granzyme B-positive and perforin-positive CTLs was observed in subject 1 as compared to the 1st dose, whereas in subject 2, no changes were observed. SARS-CoV-2-spike protein-specific tetramer-positive CD8+ T cells were markedly increased after the 2nd dose as compared to the 1st dose.

Effect of Vaccination on T_{FH} Cells and Subsets

The T_{FH} cells are major CD4+ T helper subsets that are essential for B-cell differentiation into immunoglobulin producing plasma cells, as well as for GC formation and generation of memory B cells [20–22]. The GC is the primary site for class-switched DNA recombination and af-

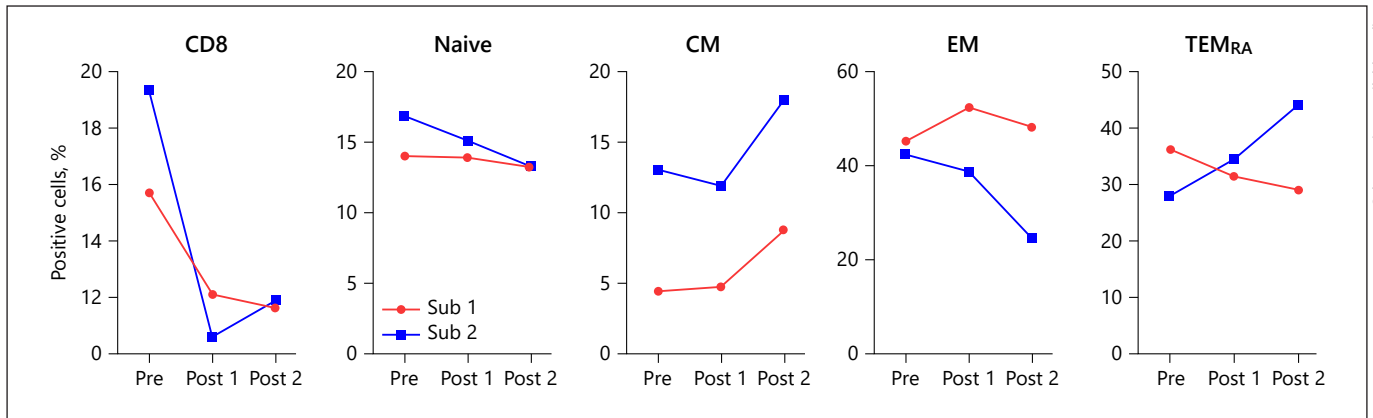


Fig. 2. Effects of vaccine on subsets of CD8+ T cells. CD8 subsets were analyzed using various markers (see Methods). T_{CM} cells were markedly increased following 2nd vaccination; changes in T_{EM} and T_{EMRA} were different among 2 subjects. T_{CM} , central memory T cells; T_{EM} , effector memory T cells; T_{EMRA} terminally differentiated effector memory T cells.

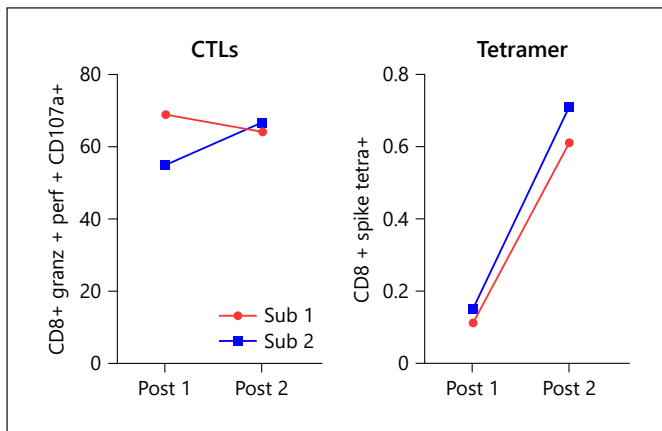


Fig. 3. Effects of vaccine on SARS-CoV-2-specific CD8+ T cells and functional CTL CD8+ T cells. SARS-CoV-2 spike protein tetramer-specific CD8+ T cells were markedly increased after the 2nd dose of vaccination. Changes in functional CTL (CD107a+ granzyme+ perforin+) cells were different among 2 subjects. CTLs, cytotoxic CD8+ T cells.

finity maturation. T_{FH} cells in the GC regulate class-switched DNA recombination and selection of high-affinity antibody-producing B cells. According to the expression of CXCR3 and CCR6 on CD4+CXCR5+ T_{FH} cells, they are divided into 3 different subsets of T_{FH} cells with different functions [23]. They include T_{FH1} , T_{FH2} , T_{FH1}/T_{FH17} , and T_{FH17} ; all are able to efficiently induce antibody response by memory B cells. We observed that T_{FH} , T_{FH1} , and $T_{FH1}/17$ cells were increased following the 1st dose, and

T_{FH1} returned to original levels after the 2nd vaccination (Fig. 4). No changes were observed in T_{FH17} cells.

Effect of Vaccination on B Cells and B-Cell Subsets

B-cell development initiates in the bone marrow, and activation, proliferation, and differentiation occur in the peripheral lymphoid tissues, including the lymph nodes and spleen [24–26]. Immature B cells leave the bone marrow as transitional B cells. A major population of transitional B cells migrate to lymphoid follicles, and a minor population migrates to the MZ. In the follicle, antigen binding to the B-cell receptor activates B cells. These antigen-activated B cells interact with T_{FH} cells, and follicular dendritic cells, where they undergo proliferation and form GCs. In the GCs, B cells undergo immunoglobulin class-switched recombination and selection of high-affinity antibody producing B cells. Subsequently, B cells leave the GCs to differentiate into long-lived plasma cells and home into the bone marrow to produce antibodies of different isotypes and subclasses. A small population of GC B cells leave the GCs to become CSM B cells. The MZ B cells after interacting with antigens differentiate into short-lived antibody-secreting plasmablasts, and a small population is retained as IgM memory B cells. We examined all these subsets of B cells.

In both subjects, MZ B cells and CD21^{low} B cells were increased, and plasmablasts were decreased following the 2nd dose (Fig. 5). However, changes in transitional B cells, CSM B cells, GC, and plasmablasts were different among both subjects. In subject 1, seroconversion following the 2nd dose was associated with increased plasmablasts.

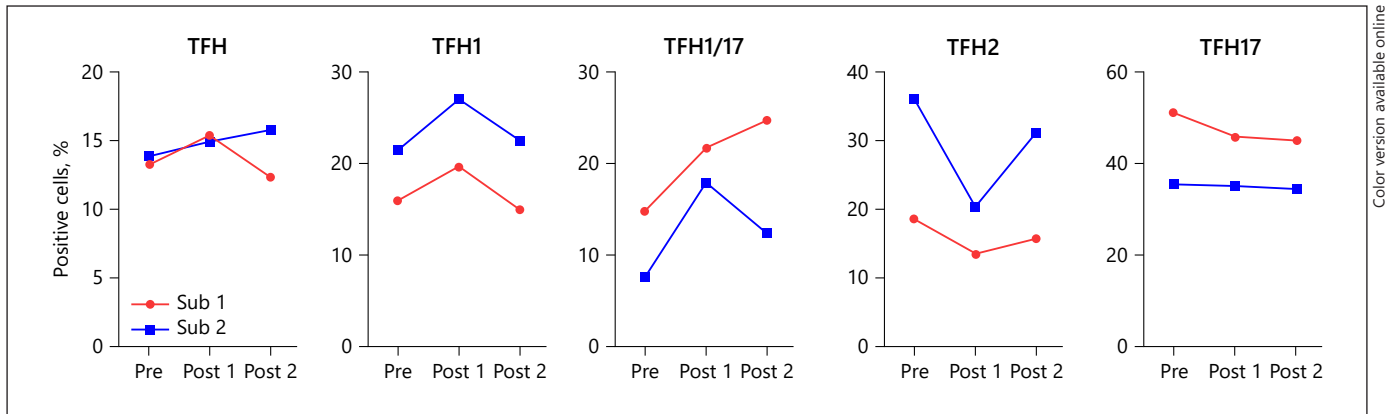


Fig. 4. T_{FH} cells' response to vaccine: T_{FH} , T_{HF1} , T_{FH2} , T_{FH17} , and $T_{FH1/17}$ were measured using a combination of surface markers. T_{FH} , $T_{FH1/17}$, and T_{FH1} were increased following the 1st dose. No effect was observed on T_{FH17} cells. T_{FH} , follicular helper T cells.

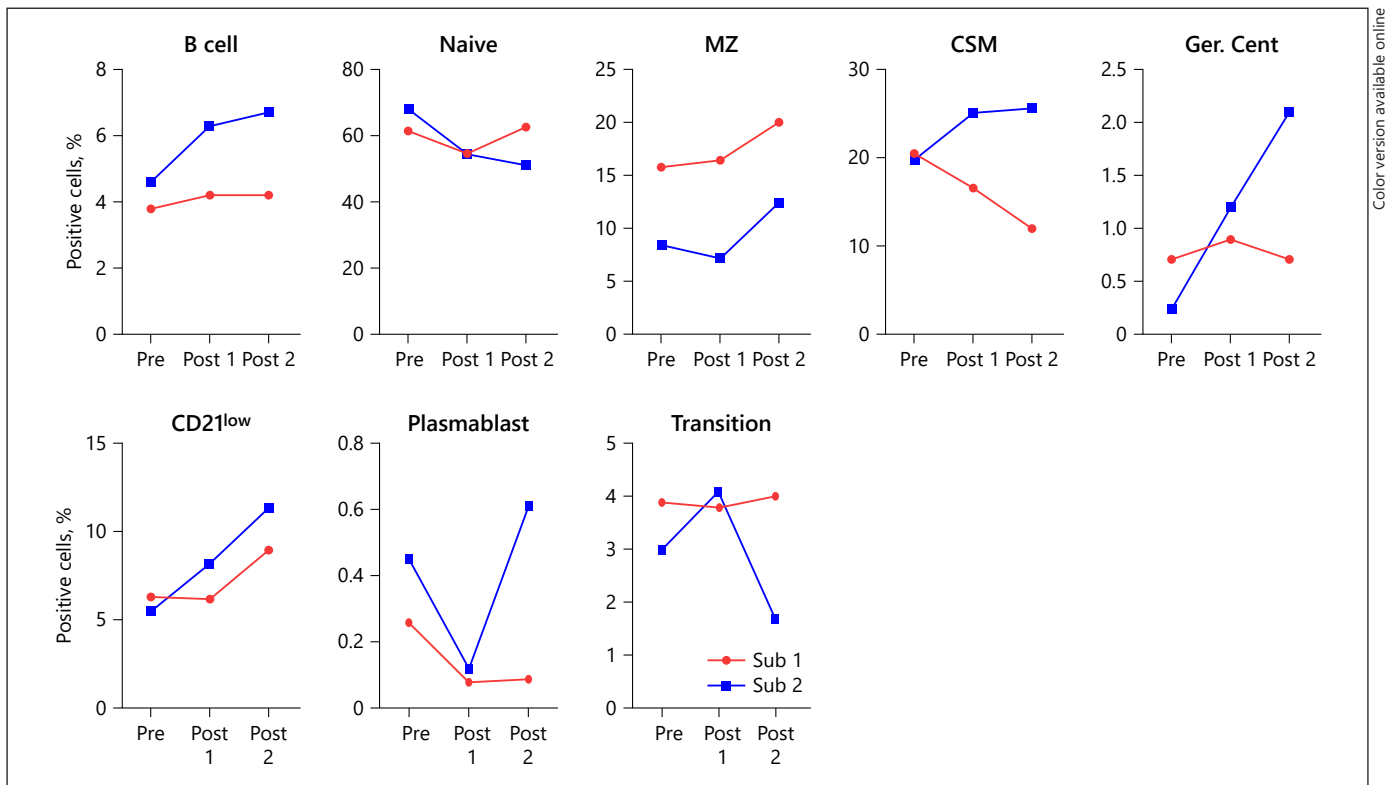


Fig. 5. Response of B cells to vaccine: MZ B cells and $CD21^{low}$ B cells were increased, whereas plasmablasts were decreased following the 2nd dose. In subject 1, plasmablasts increased when this subject developed COVID-19 antibodies. MZ, marginal zone.

Effect of Vaccination on Regulatory Lymphocytes

The members of regulatory lymphocyte club include $CD4^+$ Treg, $CD8^+$ Treg, T_{FR} , and Breg [27–34]. They play an important role in immune homeostasis. All 4 members

were evaluated (Fig. 6). In both subjects, following the 1st dose, $CD4^+$ Treg, $CD8^+$ Treg, and T_{FR} cells were increased following the 1st dose. However, following the 2nd dose, responses of $CD4^+$ Treg and $CD8^+$ Treg were different

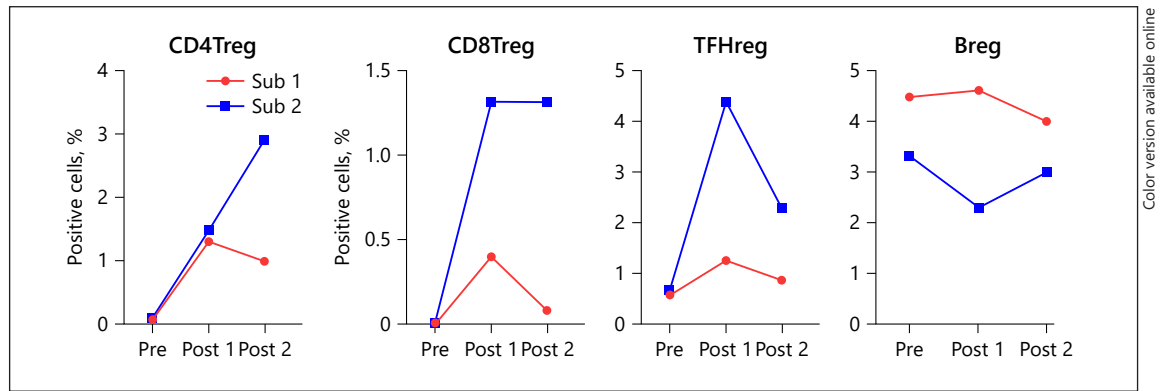


Fig. 6. Changes in regulatory lymphocytes after vaccination. CD4⁺ Treg, CD8⁺ Treg, and T_{FR} were increased following the 1st dose of vaccine. After 2nd vaccination, T_{FR} decreased when plasmablasts increased and COVID-19 antibodies became positive (subject 1). Breg were essentially unchanged.

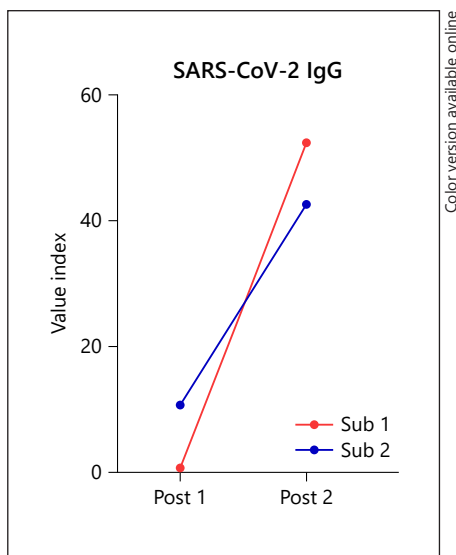


Fig. 7. SARS-CoV-2-specific IgG antibodies following vaccination. SARS-CoV-2 spike 1-specific IgG antibodies were measured 3 weeks after the 1st dose and 4 weeks after the 2nd dose by the chemiluminescent assay (semiquantitative). The VI of <0.99 is considered negative, and the VI of ≥ 1.0 is considered positive. Subject 1 had equivocal titers. Titers in both subjects were markedly increased following the 2nd dose. VI, value index.

among 2 subjects. T_{FR} cells decreased after the 2nd dose. Breg remained relatively stable.

SARS-CoV-2 Spike-Specific IgG Antibodies

IgG antibodies specific to SARS-CoV-2 spike protein 1 were measured by semiquantitative chemiluminescent

immunoassay performed on the ADVIA Centaur XPT platform, prior to vaccination, 3 weeks following the 1st dose, and 4 weeks following the 2nd dose at the same time when lymphocyte studies were performed. Data are expressed as an index value (IV). An IV < 0.99 is considered negative, and an IV ≥ 1.0 is considered positive. Data are shown in Figure 7. Subject 1 had an equivocal result (IV = 0.99) following the 1st dose; however, subject 1 made a strong antibody response following the 2nd dose (IV = 50.4). Subject 2 had a positive antibody response (IV = 3.8) following the 1st dose that was further increased (IV = 42.9) following the 2nd dose of vaccine. These data reinforce the need for the 2nd dose to obtain a strong antibody response.

Discussion

We present the 1st comprehensive study in 2 healthy subjects of the effect of SARS-CoV-2 vaccination on immune responses. Although few immunological responses were reported in vaccine clinical trials, no comprehensive cellular immunological data were published, and majority of the data were specifically for neutralizing antibodies. Sahin et al. [1] reported a 3.5-fold increase in neutralizing the antibody response at a high dose of vaccine as compared to only 0.7-fold increase with the low dose COVID-19 vaccine on day 43. This observation may be of help to use different vaccine doses in designing vaccine trials in children and adolescents. Xia et al. [35] and Zhang et al. [36] reported induction of antibody responses to inactivated SARS-

CoV-2 vaccine, BBIBP-CorV, at different doses in all vaccine recipients on day 42. Furthermore, 2-dose immunization induced higher neutralizing antibody responses than a single immunization. Our subject 1 had equivocal IgG antibody response 3 weeks post the 1st dose; however, subject 1 was positive at 4 weeks after the 2nd dose (42nd day following the 1st dose). We did not examine titers of neutralizing versus non-neutralizing antibodies. These data would suggest that the 2nd dose of corona virus vaccine is required to achieve high levels of specific antibodies. This may explain approximately lower 70% protection by the single dose Johnson & Johnson vaccine. The equivocal antibody response after 1st vaccination in our case was associated with an increase in GC cells but decreased plasmablasts. An increase in CD8+ Treg might explain decreased plasmablasts and decreased antibody production without suppressing GC cells. We have reported that CD8+ Treg inhibits plasmablasts without affecting other B-cell subsets including GC [37] and inhibit immunoglobulin production [38]. This cannot be sole mechanisms since subject 2 was antibody positive after the 1st dose and had increase in CD8 Treg; however, increase in CD8 Treg in subject 2 was modest as compared to that in subject 1. T_{FR} cells also regulate antibody production [34], and therefore, an increased T_{FR} might have also contributed to equivocal antibody response following the 1st vaccination in subjects 1. A decrease in T_{FR} cells following the 2nd vaccination might have contributed to positive antibody responses associated with further increase T_{FH} cells, and GC cells. Subject 2 had no appreciable changes in T_{FR} and CG B cells. These data demonstrate a possible role of T_{FR} in the generation of SARS-CoV-2-specific antibodies.

There are limited data on T cells from clinical trials of current vaccines. Anderson et al. [3] reported that in the clinical trial of the Moderna vaccine (SARS-CoV-2 mRNA-1273), older adults (71 years and older) had induction of T_{H1} CD4+ T-cell responses in response to S-specific peptide pool characterized by increased cytokines, IL-2 > TNF- α > IFN- γ . T_H-2 responses (IL-4 and IL-13) were unaffected. Sahin et al. [1] also reported skewed T_{H1} responses in both CD4+ and CD8+ T cells induced with the receptor binding domain peptide. In our subject, we did an extensive analysis of both CD4+ and CD8+ T cells. Analysis of CD8+ T cells and subsets in our subject revealed interesting and somewhat unexpected results. Following the 1st vaccination, the percentage of total CD8+ T cells was severely decreased. Following the 2nd vaccination, a partial recovery of total CD8+

T cells was associated with increased CD8+ T_{CM} cells. The mechanism of marked decrease in total CD8+ T cells is unclear. Dan et al. [15] also reported decreased memory CD8+ T cells in subjects who had recovered from COVID-19. CTLs are known to play an important and critical role in the elimination of viruses, therefore potentially influencing the clinical course of COVID-19. In our subject, SARS-CoV-2 spike protein-specific tetramer-positive CD8+ T cells were markedly increased following the 2nd dose as compared to the 1st dose. Functional CTL (CD107a+ granzyme B+ and perforin+) CD8 T cells were increased in subject 1 and remained unchanged in subject 2. Therefore, these data demonstrate development of SARS-CoV-2-specific CD8+ T cells, even in the absence of antibody response (subject 1). T_{CM} and T_{EM} cells are localized in lymphoid and non-lymphoid tissues, respectively. T_{CM} cells respond to antigen presented by dendritic cells in the spleen and lymph nodes, whereas T_{EM} cells mediate effector functions at extra-lymphoid sites [16, 19]. The increase in CD8 T_{CM} cells and CD4 T_{EM} cells following the 2nd vaccination may provide critical immune responses at lymphoid and non-lymphoid tissues, respectively.

Next, we compared our vaccine data with published data for SARS-CoV-2-infected COVID-19 individuals who had mild disease and/or recovered from COVID-19, to assess the effect of vaccination versus natural infection, on immune responses. SARS-CoV-2-specific T cells have been identified in T_{CM}, T_{EM}, and T_{EMRA} subsets [9]. There are limited studies of various subsets including naïve, and memory subsets of CD4+ T cells and CD8+ T cells in COVID-19. Zang et al. [13] compared T-cell subsets in severe and mild-to-moderate COVID-19 cases with healthy controls. They observed no difference in CD8+ T_N cells among 3 groups; however, both groups of patients exhibited increased percentages of CD8+ T_{EM} cells as compared to healthy controls. Furthermore, CD8+ T_{EMRA} were higher in severe cases than CD8+ T_{EMRA} in mild-to-moderate COVID-19 cases and healthy controls. Mathew et al. [39] also reported increased CD8+ T_{EMRA}; however, they observed decreased CD8+ T_{EM} cells in recovered patients as compared to healthy controls. Both our subjects had increase in CD8 T_{CM} cells. However, changes in CD8+ T_{EM} and CD8+ T_{EMRA} were different among both subjects following 2nd vaccination; subject 1 had increased CD8+ T_{EMRA} and subject 2 had increased CD8+ T_{EM}.

Mathew et al. [39] reported decreased CD4+ T_N and increased CD4+ T_{EMRA} in recovered patients as compared to healthy controls. Zhang et al. [13] also observed

increased CD4⁺ T_{EMRA} cells; however, no changes were observed in CD4⁺ T_N cells. Our subjects had increased CD4⁺ T_{EM} and decreased CD4⁺ T_{EMRA}. Therefore, CD4⁺ T cell responses following corona virus vaccination appear to differ from those of natural infection. A number of factors may have contributed to these differences, including the timing of tests performed in relation to infection and the dose of virus.

The T_{FH} cells are major CD4⁺ T helper subsets that are essential for B-cell differentiation into immunoglobulin-producing plasma cells, and for GC formation, the primary site for class switched DNA recombination, and affinity maturation [21–23]. According to secretion of the cytokines and expression of chemokines receptors, T_{FH} cells have been divided into several subtypes. T_{FH1}, T_{FH2}, T_{FH1}/T_{FH17}, and T_{FH17} are able to efficiently induce antibody response by memory B cells. Thevarajan et al. [40] observed the appearance of plasmablasts and T_{FH} cells at 7 days of SARS-CoV-2 infection peaking at day 8 and were present during convalescence. These changes were associated with the development of IgM and IgG anti-SARS-CoV-2 antibodies. Mathew et al. [39] observed increased activated (ICOS⁺) T_{FH} cells but observed no difference in PD1⁺ T_{FH} cells in COVID-19 patients. In the present subject, we also observed increased T_{FH1} cells following the 1st dose; however, they returned to the baseline level following the 2nd dose. Gupta et al. [41] reported no significant changes in T_{FH17} cells in an immunocompetent patient with mild COVID-19 disease. We also did not observe appreciable changes in T_{FH17} cells following vaccination. The changes in other subsets of T_{FH} cells were variable among 2 subjects.

Mathew et al. [39] reported decreased naïve and CSM B cells, and increased non-class-switched IgM memory B cells, transitional B cells, and antibody secreting cells (plasmablasts) in recovered patients as compared to healthy controls. There are no detailed data on various B-cell subsets following vaccination. In both our subjects following the 2nd vaccination, an increase in MZ B cells and CD21^{low} was observed. In subject 1, the status of COVID-19 antibodies correlated with circulating plasmablasts and decreased following the 1st dose of vaccine when COVID-19 antibodies were equivocal, and plasmablasts increased when the subject became antibody-positive following the 2nd dose of vaccine.

CD21 forms a complex with CD19 and CD81 to act as a B-cell co-receptor. This population of B cells is distinct from other B-cell subpopulations as it resembles innate-like B cells [42]. Woodruff et al. [43] observed increased CD21^{low} B cells in mild cases of COVID-19. In our sub-

ject, CD21^{low} B cells were also increased following vaccination.

Regulatory lymphocytes play a critical role in immune homeostasis and immunological tolerance [28, 30, 31, 33]. However, Zhang et al. [13] did not find any difference in CD4⁺ Treg among mild, severe cases, and healthy controls. However, in our subjects, CD4⁺ Treg cells were increased following vaccination. There are no published data on CD8 Treg, Breg, and T_{FR} cells in mild COVID-19 disease. We observed an increase in both CD8 Treg and TFR after the 1st dose. In subject 1, T_{FR} decreased following the 2nd dose that correlates with the increase in GC cells, appearance of plasmablasts, and positive COVID-19 antibodies. Therefore, TFR may play a role in COVID-19 production following vaccination.

In summary, our data clearly demonstrate that the 2nd dose of mRNA vaccine is required to obtain high levels of T- and B-cell immune responses. Our data also show that vaccine could induce SARS-CoV-2-specific T-cell responses before the development of antibody response. Furthermore, regulatory lymphocytes, especially T_{FR}, may play an important role in the generation of SARS-CoV-2-specific antibodies. Finally, our study suggests that immune responses following vaccination may be different from those from natural infections. One of the factors for heterogeneity of certain immune responses to mRNA COVID-19 vaccine in our 2 subjects could be age difference. However, that appears unlikely because immune responses to vaccine were better in subject 1 (76 years) than in subject 2 (52 years), suggesting that vaccine may be equally effective in aged subjects.

The main limitation of the study is the analysis on 2 men. Therefore, this study does not address whether vaccine responses will be different between male and female, and if the vaccine responses in younger individuals (18–50 years) would be different from those in older subjects. Baden et al. [2] observed similar efficacy of the mRNA-1273 SARS-CoV-2 vaccine between males and females. However, because of the small number of cases, authors were unable to evaluate the efficacy of the vaccine in aged individuals and different ethnic groups. A phase I study of the Moderna vaccine observed that antibody responses in older subjects were similar to those seen in younger people [3]. However, a number of older subjects were small. Moderna mRNA vaccine showed a 95.6% virus efficiency (VE) for people younger than 65 years, but the VE dropped to 86.4% for people older than 65 years. Pfizer mRNA vaccine showed a VE of 94.7% in individuals older than 65 years, which is comparable to that observed in younger

population [44]. Therefore, comprehensive studies similar to ours are needed in a large cohort of individuals to address some of these limitations.

Statement of Ethics

The study was conducted according to guidelines of the Declaration of Helsinki and approved by the IRB Committee (Human) of the University of California, Irvine (Protocol number H.S.: 2001-2072). Both subjects gave written informed consent. The consent form includes a statement of permission to publish.

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- 1 Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M. COVID-19 vaccine BNT162b1 elicits human antibody and T(H)1 T cell responses. *Nature*. 2020 Oct;586(7830): 594–9.
- 2 Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021 Feb 4;384(5):403–16.
- 3 Anderson EJ, Roupheal NG, Widge AT, Jackson LA, Roberts PC, Makhene M, et al.; mRNA-1273 Study Group. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N Engl J Med*. 2020; 383(25):2427–38.
- 4 Rha MS, Jeong HW, Ko JH, Choi SJ, Seo IH, Lee JS, et al. PD-1-expressing SARS-CoV-2-specific CD8+ T cells are not exhausted, but functional in patients with COVID-19. *Immunity*. 2021;54(1):44–52.e3.
- 5 de Candia P, Prattichizzo F, Garavelli S, Matarese G. T cells: warriors of SARS-CoV-2 infection. *Trends Immunol*. 2021;42(1):18–30.
- 6 Ni L, Ye F, Cheng ML, Feng Y, Deng YQ, Zhao H, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. *Immunity*. 2020;52(6):971–77.e3.
- 7 Weiskopf D, Schmitz KS, Raadsen MP, Grifoni A, Okba NMA, Endeman H, et al. Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Sci Immunol*. 2020 Jun 26;5(48):eabd2071.
- 8 Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. 2020 May 1;130(5): 2620–9.
- 9 Rha MS, Jeong HW, Ko JH, Choi SJ, Seo IH, Lee JS, et al. PD-1-expressing SARS-CoV-2-specific CD8+ T cells are not exhausted, but functional in patients with COVID-19. *Immunity*. 2021;54(1):44–52.e3.
- 10 Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol*. 2020;11: 827.
- 11 Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell*. 2020;181:1489–501.e15.
- 12 Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. *Cell*. 2020 Oct 1;183(1):158–68.e14.
- 13 Zhang F, Gan R, Zhen Z, Hu X, Li X, Zhou F, et al. Adaptive immune responses to SARS-CoV-2 infection in severe versus mild individuals. *Signal Transduct Target Ther*. 2020 Aug 14;5(1):156. Erratum in: *Signal Transduct Target Ther*. 2021 Apr 19;6(1):161.
- 14 Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med*. 2020 Jun;26(6):845–8.
- 15 Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. 2021 Feb 5;371(6529): eabf4063.
- 16 Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401:708–12.
- 17 Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. *Blood*. 2003;101:4260–6.
- 18 Van Lier RA, ten Berge IJ, Gamadia LE. Human CD8(+) T-cell differentiation in response to viruses. *Nat Rev Immunol*. 2003;3: 931–9.
- 19 Gupta S. Molecular mechanisms of TNF- α -induced apoptosis in naïve and memory T cell subsets: effect of age. *Immunol Rev*. 2005;205: 114–25.
- 20 Ueno H. Human circulating T follicular helper cell subsets in health and disease. *J Clin Immunol*. 2016 May;36 Suppl 1:34–9.
- 21 Victora GD, Schwickert TA, Fooksman DR, Kamphorst AO, Meyer-Hermann M, Dustin ML, et al. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell*. 2010 Nov 12;143(4):592–605.
- 22 Crotty ST. Follicular helper cell biology: a decade of discovery and diseases. *Immunity*. 2019 May 21;50(5):1132–48.
- 23 Morita R, Schmitt N, Bentebibel SE, Ranganathan R, Bourdery L, Zurawski G, et al. Human blood CXCR5(+)CD4(+) T cells are counterparts of T follicular cells and contain specific subsets that differentially support antibody secretion. *Immunity*. 2011 Jan 28;34(1):108–21. Erratum in: *Immunity*. 2011 Jan 28;34(1): 135.
- 24 LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood*. 2008 Sep 1; 112(5):1570–80.
- 25 Kurosaki T. B-lymphocyte biology. *Immunol Rev*. 2010 Sep;237(1):5–9.
- 26 Martin F, Kearney JF. Marginal-zone B cells. *Nat Rev Immunol*. 2002 May;2(5):323–35.

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Author Contributions

H.S. performed flow cytometry. S.A. supervised H.S. and analyzed the data, S.G. conceived the idea, designed the study, and wrote the manuscript.

Data Availability Statement

Data are stored in files located in research laboratories and will be provided on request.

- 27 Damle NK, Gupta S. Heterogeneity of concanavalin A-induced suppressor T cells in man defined with monoclonal antibodies. *Clin Exp Immunol*. 1982 Jun;48(3):581–8.
- 28 Sakaguchi S, Miyara M, Costantino CM, Haffler DA. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol*. 2010;10(7):490–500. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20559327>.
- 29 Rifa'i M, Kawamoto Y, Nakashima I, Suzuki H. Essential roles of CD8+CD122+ regulatory T cells in the maintenance of T cell homeostasis. *J Exp Med*. 2004 Nov 1;200(9):1123–34.
- 30 Flippe L, Bézie S, Anegón I, Guillonnet C. Future prospects for CD8+ regulatory T cells in immune tolerance. *Immunol Rev*. 2019 Nov;292(1):209–24.
- 31 Ding T, Yan F, Cao S, Ren X. Regulatory B cell: new member of immunosuppressive cell club. *Hum Immunol*. 2015 Sep;76(9):615–21.
- 32 Filatreau S. Regulatory functions of B cells and regulatory plasma cells. *Biomed J*. 2019 Aug;42(4):233–42.
- 33 Mauri C, Bosma A. Immune regulatory function of B cells. *Annu Rev Immunol*. 2012;30:221–41.
- 34 Zhu Y, Zou L, Liu YC. T follicular helper cells, T follicular regulatory cells and autoimmunity. *Int Immunol*. 2016 Apr;28(4):173–9.
- 35 Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect Dis*. 2021 Jan;21(1):39–51.
- 36 Zhang Y, Zeng G, Pan H, Li C, Hu Y, Chu K, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18–59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect Dis*. 2021 Feb;21(2):181–92.
- 37 Gupta S, Su H, Agrawal S. CD8 Treg cells inhibit B-cell proliferation and immunoglobulin production. *Int Arch Allergy Immunol*. 2020 Aug 14;181:947–55.
- 38 Gupta S, Agrawal S. In vitro effects of CD8+ regulatory T cells on human B cell subpopulations. *Int Arch Allergy Immunol*. 2020;181(6):476–80.
- 39 Mathew D, Giles JR, Baxter AE, Oldridge DA, Greenplate AR, Wu JE, et al. Deep immune profiling of COVID-19 patients reveals distinct immunotypes with therapeutic implications. *Science*. 2020 Sep 4;369(6508):eabc8511.
- 40 Thevarajan I, Nguyen THO, Koutsakos M, Druce J, Caly L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nat Med*. 2020 Apr;26(4):453–5.
- 41 Gupta S, Su H, Narsai T, Agrawal S. SARS-CoV-2-associated T-cell responses in the presence of humoral immunodeficiency. *Int Arch Allergy Immunol*. 2021;182(3):195–209.
- 42 Rakhmanov M, Keller B, Gutenberger S, Foerster C, Hoening M, Driessen G, et al. Circulating CD21low B cells in common variable immunodeficiency resemble tissue homing, innate-like B cells. *Proc Natl Acad Sci U S A*. 2009 Aug 11;106(32):13451–6.
- 43 Woodruff MC, Ramonell RP, Nguyen DC, Cashman KS, Saini AS, Haddad NS, et al. Extrafollicular B cell responses correlate with neutralizing antibodies and morbidity in COVID-19. *Nat Immunol*. 2020 Dec;21(12):1506–16.
- 44 Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al.; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020 Dec 31;383(27):2603–15.