Brief Report: Humoral and cellular immune responses to SARS-CoV-2 infection and vaccination in B cell depleted autoimmune patients

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Abstract

Objective. B cell depletion is an established therapeutic principle in a wide range of autoimmune disease. However, B cells are also critical for inducing protective immunity after infection and vaccination. We therefore assessed humoral and cellular immune responses after infection with or vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in B cell depleted patients and B cell competent healthy controls.

Methods. Antibody (ELISA) and T cell (IFNγ ELISPOT) responses against the SARS-CoV-2 spike S1 and nucleocapsid proteins were assessed in a limited number of infected (N=6) and vaccinated (N=8) B cell depleted autoimmune patients as well as infected (N=30) and vaccinated (N=30) healthy controls.

Results. As expected, B and T cell responses to the nucleocapsid were observed only after infection, while respective responses to spike S1 were found both after infection and vaccination. A SARS-CoV-2 antibody response was observed in all vaccinated controls (30/30, 100%) but in none (0/8) of the vaccinated B-cell-depleted patients. In contrast, after SARS-CoV-2 infection, both B-cell-depleted patients (spike S: 5/6, 83%; nucleocapsid 3/6, 50%) and healthy controls (spike S: 28/30, 94%; nucleocapsid 28/30, 93%) developed antibodies. T cell responses against the spike S1 and nucleocapsid proteins were found in both infected and vaccinated B cell depleted subjects and in the controls.
Conclusion. These data show that B cell depletion completely blocks humoral but not T cell SARS-CoV-2 vaccination response. Furthermore, limited humoral immune responses are found in B cell depleted patients after SARS-CoV-2 infection.

Introduction

Depletion of B cells is an effective therapeutic strategy to treat severe autoimmune disease (1). Diseases with robust activation of B cells and plasma cells, such as rheumatoid arthritis (2), multiple sclerosis (3), granulomatosis with polyangiitis (4), dermatomyositis (5), IgG4 syndrome (6), pemphigus (7) and immune thrombocytopenic purpura (8) are sensitive to B cell targeting. Rituximab, a monoclonal antibody binding the B cell specific surface molecule CD20, effectively depletes circulating B cells over a period of several months and shows widespread therapeutic efficacy in patients with autoimmune disease (9). B cell depletion, however, may also seriously impair the development of protective immunity after infection and vaccination. Of note, rituximab treatment has been associated with more severe courses of COVID-19 (10) and impaired immune response to established vaccines (11,12). To date, reliable data on the impact of B-cell depletion on the dynamics of protective antibody responses upon infection and vaccination remain sparse (13), while protective antibody responses have been clearly documented in naïve (14) and previously infected (15) immune competent subjects. The current SARS-CoV-2 pandemic thus provides an unique opportunity to profile the immune response of a naïve population to a defined infectious agent. In addition, it permits to study quality and quantity of a newly evolving adaptive immune response to infection and to vaccination in both healthy individuals and in B-cell depleted patients, respectively.
Methods

Ethical Approval

Ethical approval (#157_20 B) to conduct this analysis was granted by the institutional review board of the University Clinic of Erlangen as the responsible ethics committee. Written informed consent was obtained from the study participants.

Patients and Controls

Sera of rituximab-treated patients and healthy controls were collected within the COVID-19 study program of the Deutsche Zentrum fuer Immuntherapie (DZI) (16). This study program has been initiated in February 2020 and monitors anti-SARS-CoV-2 antibody responses in healthy controls, COVID-19 patients and autoimmune patients (16). Healthy controls did not have an immune-mediated inflammatory disease nor did they receive any treatment with immune-modulatory agents. Vaccinated patients and vaccinated healthy controls did not have any history of COVID-19 or positive COVID-19 PCR before the analysis.

Rituximab-treated patients and healthy controls were vaccinated with BNT162b2 mRNA SARS-CoV-2 vaccine at official public vaccination centers based on occupational exposure risk, comorbidities and age-related risk in accordance with the recommendations of the Robert Koch Institute (RKI). Sera were collected at least 10 days after the second vaccination and at least 30 days after onset of infection in the infected participants. In all participants we collected demographic data (age, sex) as well as disease-specific data (type of autoimmune disease, type of treatment). Mean elapsed time between antibody testing and infection (3.8 months; range 1-8; SD 2.9) or vaccination (3.6 months; range 1-8; SD 3.0), respectively, was very similar in the rituximab-treated patients. Mean number of rituximab infusions was 5.4±4.3, administered at a dose of 1000 mg every 6 months.
Anti-SARS-CoV-2 antibodies

IgG antibodies against the S1 domain of the spike protein and nucleocapsid protein of SARS-CoV-2 were tested by two CE commercial enzyme-linked immunosorbent assay (Euroimmun, Lübeck, Germany; Epitope Diagnostics, USA) according to the manufacturers protocols. Optical density was determined at 450 nm with reference wavelength at 630 nm. A cutoff of <0.8 and <0.2 was considered as negative for IgG antibodies against spike S1 protein and nucleocapsid, respectively. An in-house neutralization assay for assessment of inhibition of binding to ACE2 by antibodies was used. Assays were performed in line with the guidelines of the German Medical Association (RiliBAK) with stipulated internal and external quality controls.

Anti-SARS-CoV-2 T cells

The detection of SARS-CoV-2 specific T-cells was conducted via a γ-IFN ELISpot Assay (T-SPOT.COVID, Oxford Immunotec). Isolation of peripheral blood mononuclear cells (PBMCs) was carried out via density gradient centrifugation. Leucosep™tubes (Greiner Bio One GmbH, Frickenhausen, Germany) were filled with 15ml Lymphoflot (Bio-Rad Laboratories GmbH, Feldkirchen, Germany) and centrifuged briefly to collect the fluid under the membrane. A maximum of 30ml citrate blood was transferred to the tube and filled up to 50ml with RPMI 1640 medium (Gibco, Carlsbad, California, United States) pre-warmed to 37°C. Cells were centrifuged at 760xg for 20min and the upper layer containing PBMCs was transferred to 50ml tubes and centrifuged at 610xg for 10min. The cell pellet was then washed with 30ml 37°C-warm RPMI 1640 medium at 610xg for 10min prior to resuspension at a concentration of 2.5x10⁶/ml in AIM-V medium (Gibco, Carlsbad, California, United States) pre-warmed to 37°C. 50µl of either AIM-V medium, Panel A, Panel B or Positive Control were added to the wells of the pre-coated multititer ELISpot plate (Oxford Immunotec). 100µl of the cell suspension was added to each well and carefully mixed by pipetting. After an incubation period at 37°C and 7% CO₂ for 16-20h, the wells were washed four times with 200µl PBS (Gibco, Carlsbad, California, United States). The conjugate reagent was diluted 1:200 in PBS and 50µl of this dilution was added to each well. Following a 60min incubation period at 4°C, the wells were washed four times with 200µl PBS. 50µl substrate solution was added to each well and incubated for 7min. The plate was washed three times with H₂O and then air-dried. The spots were counted and analyzed using an ELISpot reader (AID, Strassberg, Germany). Results are reported as SFUs (Spot forming units) per 2.5x10⁵ cells. According to the manufacturer’s guidelines, a response was considered positive when the number of...
spots in the respective panel was \( \geq 8 \) SFUs above the negative control. Samples with negative controls \( > 10 \) SFUs were considered invalid.

Statistical analysis

Subject characteristics were summarized using means and standard deviations for continuous data and counts and percentages for categorical data. We used Wilcoxon’s rank-sum test for pairwise between-group comparisons of optical density from the anti-spike S1 IgG and anti-nucleocapsid IgG assays. P values were adjusted for a family of 6 possible pairwise comparisons per assay using the Bonferroni-Holm method and considered significant when <0.05.
Results

To address the question, whether autoimmune patients depleted from peripheral B cells are able to develop specific humoral immunity to SARS-CoV-2 vaccination, we screened data from an ongoing longitudinal SARS-CoV-2 antibody study in Germany, which measures IgG responses against the SARS-CoV-2 spike S1 protein (Euroimmun, Lübeck, Germany) and the nucleocapside (Epitope Diagnostics, USA) in patients with autoimmune inflammatory diseases and healthy controls (16). We identified 8 rituximab-treated patients that received the BNT162b2 mRNA SARS-CoV-2 vaccine and 6 rituximab-treated patients that had undergone clinically symptomatic, mRNA-proven infection with SARS-CoV-2. The most frequent COVID-19 related symptoms in 6 rituximab-treated patients with SARS-CoV-2 infection were cough (N=5), anosmia (N=5), fever (N=4) and dyspnea (N=4). Three patients required hospitalization, none of them required intensive care. Characteristics of patients and controls are summarized in Table 1.

All 14 rituximab-treated vaccinated or infected patients were tested for anti- SARS-CoV-2 IgG antibodies after having received their second shot of the vaccine or at least 4 weeks after the infection, respectively. For control purposes, anti- SARS-CoV-2 IgG antibodies were also tested in 30 healthy controls after SARS-CoV-2 vaccination and 30 additional healthy controls after SARS-CoV-2 infection. The majority (93%) of SARS-CoV-2 infected (mean±SD OD450nm: 5.4±2.5; cutoff positive >0.8) and all (100%) vaccinated (8.1±2.5) controls developed IgG antibodies against the spike S1 protein (Figure 1A). As expected, IgG antibodies against the nucleocapsid were observed only in infected (mean±SD 0.31±0.09; cutoff positive >0.2) but not in vaccinated (0.10±0.04) controls.

Although anti- SARS-CoV-2 S1 IgG levels were lower than that in healthy controls, 5 out of 6 (83.3%) rituximab-treated SARS-CoV-2 infected patients surprisingly developed IgG antibodies (2.9±2.2) (Figure 1A). These antibodies in rituximab-treated infected patients also had similar neutralizing capacity as those of infected controls. In contrast, none of the 8 SARS-CoV-2 vaccinated rituximab-treated patients developed anti-SARS-CoV-2 IgG antibodies (0.2±0.3). Mean elapsed time between antibody testing and infection (3.8 months; range 1-8; SD 2.9) or vaccination (3.6 months; range 1-8; SD 3.0), respectively, was very
similar in the rituximab-treated patients. In addition, time interval between the last rituximab infusion and infection/vaccination was comparable (infection: 2.9±3.8 months; vaccination: 3.1±3.7 months).

Peripheral B cells were undetectable or lower than 15 cells/microliter in all infected and vaccinated patients. Also, Peripheral CD4 and CD8 T cell counts as well as serum levels of immunoglobulins IgG, IgA and IgM did not differ between infected and vaccinated rituximab-treated patients (Figure 1B). We were also able to assess SARS-CoV-2 specific T-cell responses using an IFN-gamma ELISpot assay in a limited subset of the patients from each group (Figure 1C). T cell responses against both the spike S1 protein and the nucleocapsid were found in healthy controls and B cell depleted patients after SARS-CoV-2 infection. Furthermore, also the majority of vaccinated patients, including the ones depleted for B cells, developed a T cell response against the spike S1 protein, while, as expected no T cell responses against the nucleocapsid were found in the vaccinated patients.
Discussion

These data provide interesting and unexpected new insights into the immune response to infection and vaccination in B cell depleted patients. First, they show that SARS-CoV-2 vaccination fails to trigger significant humoral immune responses in B cell depleted patients. This finding may suggest that vaccination should preferentially take place before a B cell depleting treatment is started in order to mount a significant humoral immune response.

Surprisingly and in contrast to vaccination, infection with SARS-CoV-2 triggered specific antibody responses despite the absence of circulating B cells. Though these antibody responses are lower than in healthy controls, this finding sheds light on the differences between vaccination and infection: While in vaccination local antigen presentation and T- and B- cell activation may prevail, infection may trigger a much more systemic adaptive immune response. Hence, residual tissue B cells (e.g. in the bone marrow), which may escape rituximab treatment, could be sufficient to induce a humoral immune response after SARS-CoV-2 infection. Previous biopsy data in rituximab-treated patients, who were depleted of circulating peripheral B cells have shown that tissue B cells can escape depletion (17).

Second, our data indicate that SARS-CoV-2 vaccination, like infection, can trigger specific T cell mediated immune responses even in the absence of peripheral B cells. Such T cell responses may explain why B cell depleted patients can still control SARS-CoV-2 infection. In addition, the data also suggest that potentially protective T cell-mediated immunity may develop after the vaccination in the absence of B cells.

A limitation of this study is the limited number of B cell depleted patients exposed to SARS-CoV-2 infection and vaccination. Though results were highly consistent even in this small sample, further studies will be required. Furthermore, only BNT162b2 mRNA SARS-CoV-2 vaccine was assessed in this data set requiring the collection of additional data from other, i.e. vector-based vaccines. Despite these limitations, the data very consistently showed that T cell responses against SARS-CoV-2 can develop in both vaccinated and infected B cell depleted patients. The data also indicate that SARS-CoV-2 infection, but not vaccination can in
principle trigger limited humoral immune responses against the virus in B cell depleted patients, indicating that infection can reach and activate residual tissue B cells.

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Figure Legends

Figure 1. Anti-SARS-CoV-2 immune responses in infected and vaccinated patients having undergone B cell depletion

(A) Antibodies against the spike S1 protein (left; Euroimmun ELISA) and the nucleocapsid (middle; Epitope Diagnostics ELISA) of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Tests were done in 30 healthy controls after SARS-CoV-2 infection (CTRL-COVID-19), 30 healthy controls after SARS-CoV-2 mRNA vaccination (CTRL-VACC), 6 rituximab-treated B-cell depleted patients with autoimmune disease after SARS-CoV-2 infection (RTX-COVID19) and 8 rituximab-treated B-cell depleted patients with autoimmune disease after SARS-CoV-2 mRNA vaccination (RTX-VACC) Y-axis shows optical density (OD) at 450 nanometer. Each dot represents one patient. Comparisons were done by Wilcoxon’s signed rank-sum test. (right) correlation of neutralizing antibody activity (in % inhibition of binding of spike S1 protein expressing cells to ACE2) with anti-SARS-CoV-2 spike S1 protein IgG in infected controls and rituximab-treated patients (B) (top) serum
immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) levels and (bottom) peripheral CD19 B cell, CD4 T cell and CD8 T cell numbers in rituximab-treated SARS-CoV-2 infected (RTX-COVID19) and vaccinated (RTX-VACC) patients. (C) ELISPOT showing T cell responses against the spike S1 protein (left) and the nucleocapsid (right). Y-axis shows spot forming units (SFU). T cell responses were analyzed in healthy controls after SARS-CoV-2 infection (CTRL-COVID-19) and after SARS-CoV-2 mRNA vaccination (CTRL-VACC) as well as in rituximab-treated B-cell depleted patients after SARS-CoV-2 infection (RTX-COVID19) or vaccination (RTX-VACC).
Table 1. Characteristics of patients and controls

|                      | Healthy controls Infection | Healthy controls Vaccination | B depleted patients Infection | B depleted patients Vaccination |
|----------------------|----------------------------|------------------------------|-------------------------------|-------------------------------|
| N                    | 30                         | 30                           | 6                             | 8                             |
| Age (years), mean±SD | 61.0±16.6                  | 57.1±7.5                     | 62.5±12.8                     | 53.5±7.7                      |
| Sex (female), N (%)  | 12 (40.0)                  | 23 (76.7)                    | 5 (83.3)                      | 5 (83.3)                      |

Humoral Immune response

|                      | Healthy controls Infection | Healthy controls Vaccination | B depleted patients Infection | B depleted patients Vaccination |
|----------------------|----------------------------|------------------------------|-------------------------------|-------------------------------|
| Anti-Spike S1 IgG (OD), mean±SD | 5.4±2.5                   | 8.1±2.5                      | 2.9±2.2                       | 0.2±0.3                       |
| Anti-Spike S1 IgG >OD 0.8, N (%) | 28 (93.3)                 | 30 (100.0)                   | 5 (83.3)                      | 0 (0)                         |
| Anti-Nucleocapsid IgG (OD), mean±SD* | 0.31±0.09               | 0.10±0.04                    | 0.18±0.09                     | 0.09±0.02                     |
| Anti- Nucleocapsid IgG >OD 0.2, N (%)* | 28 (93.3)               | 0 (0)                        | 3 (50.0)                      | 0 (0)                         |

Cellular Immune response

|                      | Healthy controls Infection | Healthy controls Vaccination | B depleted patients Infection | B depleted patients Vaccination |
|----------------------|----------------------------|------------------------------|-------------------------------|-------------------------------|
| Anti-Spike S1 IFNγ (SFU) >3, N (%) | 4/5 (80)                  | 5/5 (100.0)                  | 6/6 (100.0)                   | 6/8 (75%)                     |
| Anti- Nucleocapsid IFNγ (SFU) >5, N (%) | 5/5 (100)               | 0/5 (0)                      | 5/6 (83.3)                    | 0/8 (0)                       |

Diseases

|                      | Healthy controls Infection | Healthy controls Vaccination | B depleted patients Infection | B depleted patients Vaccination |
|----------------------|----------------------------|------------------------------|-------------------------------|-------------------------------|
| Granulomatosis with polyangiitis, N (%) | -                          | -                            | 2 (33.3)                      | 3 (37.5)                      |
| Rheumatoid Arthritis, N (%) | -                          | -                            | 3 (50.0)                      | 3 (37.5)                      |
| Multiple Sclerosis, N (%) | -                          | -                            | 0 (0)                         | 1 (12.5)                      |
| Dermatomyositis, N (%) | -                          | -                            | 0 (0)                         | 1 (12.5)                      |
| IgG4 syndrome, N (%) | -                          | -                            | 1 (16.7)                      | 0 (0)                         |

IFNγ, interferon-gamma; IgG, immunoglobulin G; *Anti-Nucleocapsid IgG were only measure in 6 of the 8 vaccinated B cell depleted patients
