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Original article

Altered cellular immune response to vaccination against SARS-CoV-2 in patients suffering from autoimmunity with B-cell depleting therapy

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ABSTRACT

B-cell depleting therapies result in diminished humoral immunity following vaccination against COVID-19, but our understanding on the impact on cellular immune responses is limited. Here, we performed a detailed analysis of cellular immunity following mRNA vaccination in patients receiving B-cell depleting therapy using ELISpot assay and flow cytometry. Anti-SARS-CoV-2 spike receptor-binding domain antibody assays were performed to elucidate B-cell responses. To complement our cellular analysis, we performed immunophenotyping for T- and B-cell subsets. We show that SARS-CoV-2 vaccination using mRNA vaccines elicits cellular T-cell responses in patients under B-cell depleting therapy. Some facets of this immune response including TNFα production of $CD4^+$ T-cells and granzyme B production of $CD8^+$ T-cells, however, are distinctly diminished in these patients. Consequently, it appears that the finely coordinated process of T-cell activation with a uniform involvement of $CD4^+$ and $CD8^+$ T-cells as seen in HCs is disturbed in autoimmune patients. In addition, we observed that immune cell composition does impact cellular immunity as well as sustainability of anti-spike antibody titers. Our data suggest disturbed cellular immunity following mRNA vaccination in patients treated with B-cell depleting therapy. Immune cell composition may be an important determinant for vaccination efficacy.

The coronavirus disease 2019 (COVID-19) pandemic has urged the rapid development of countermeasures including vaccines of diverse formulations and thus facilitated the entry of mRNA vaccines on the world stage. Thereby, mRNA vaccines BNT162b2 (BioNTech-Pfizer) and mRNA-1273 (Moderna/NIAID) have demonstrated high efficacy and safety in clinical trials for COVID-19 prevention [1–3]. Vaccine-elicited protection from COVID-19 is mainly described by the concentration of antibodies binding to spike protein or receptor-binding domain (RBD) or titers of neutralizing antibodies to SARS-CoV-2 [4–6], but accumulating evidence suggests that $CD4^+$ and $CD8^+$ T-cell responses also play important roles in the resolution of SARS-CoV-2 infection and protection from COVID-19 [7]. Moreover, T-cells have a range of different functionalities beyond helping antibody responses including the production of TNFα, IL-2 or GzmB that are vital in the context of antiviral immunity [8,9]. Thus, detailed assessment of all arms of adaptive immunity is of utmost importance to gain insights into SARS-CoV-2 protective immunity [10].

Immunocompromised individuals are at an increased risk of severe COVID-19 with enhanced mortality rates and therefore are

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considered a high priority for COVID-19 vaccination [11]. In this context, the immunogenicity of vaccination during immunomodulatory therapies, such as B-cell depletion by anti-CD20 antibodies, is a major concern. It is generally well accepted that vaccinations in these patients usually only reach low efficacy [12]. In line with this notion, we recently reported that humoral immunity to COVID-19 vaccination is distinctly diminished in immunocompromised individuals in an interim analysis of the CoVVac trial (NCT04858607) [13]. Mrak et al. have recently provided some initial evidence that T-cell-mediated immune response is maintained even in the absence of a humoral anti-SARS-CoV-2 response [14], but only limited information is available on the detailed effector functions of helper and cytotoxic T-cells.

Here, we approach to address T-cell reactivity to SARS-CoV-2 vaccination in-depth and determine the effect of B-cell depleting therapy on various T-cell effector functions using data from an interim analysis of the prospective, open-label, phase IV CoVVac trial (NCT04858607).

1. Methods

1.1. Study design and participants

We report the data of an interim analysis of the CoVVac trial (NCT04858607), which is an ongoing open-label, phase IV, prospective, monocentric, interdisciplinary study at the Medical University of Graz, Austria. After approval by the ethics committee of the Medical University of Graz in April 2021 (EK 1128/2021), patients receiving B-cell-depleting therapy and age- and sex-matched healthy controls were recruited before receiving their first dose of COVID-19 vaccine. The detailed study protocol is provided in the Supplementary Information. In brief, blood was drawn before the first vaccination with BNT162b2 (BioNTech/Pfizer) or mRNA-1273 (Moderna) for peripheral blood mononuclear cell (PBMC) isolation and lymphocyte phenotyping. The second vaccination was administered 21 (BNT162b2) or 28 days (mRNA-1273) after the first one. Blood sampling was performed 21–28 days after the second vaccination to analyze the COVID-19-specific antibody and T-cellular immune responses.

1.2. Lymphocyte phenotyping

Blood samples from the baseline visit were processed within 4 h for analysis by flow cytometry. For lymphocyte phenotyping, ethylenediaminetetraacetic acid whole blood was stained for CD3, CD4, CD8, CD45, CD16, CD56, and CD19. For immune cell phenotyping, PBMCs were isolated from lithium heparin whole blood by Ficoll gradient density centrifugation. One million PBMCs were incubated with the following antibodies: CD19-VioGreen, anti-IgD-VioBlue, CD24-PerCP-Vio700, CD38-FITC, CD27-APC, CD86-PE-Vio770, CD21-APC-Vio770, and anti-IgM-PE (Miltenyi Biotec, Bergisch Gladbach, Germany). Samples were measured using a FACSLyric flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Data were analyzed using FACSSuite (BD Biosciences).

1.3. Antibody assays

Blood was obtained before the first vaccination dose, 21–28 days and 6 months after the second dose. Serum was aliquoted, frozen, and stored at −80 °C until analysis was performed in batches. Anti-SARS-CoV-2 specific Ig was determined using the Roche Elecsys anti-SARS-CoV-2 S electrochemiluminescence immunoassay targeting the receptor-binding domain of the viral spike protein using a Cobas e 801 analytical unit (Roche Diagnostics GmbH, Mannheim, Germany). Its quantification range lies between 0.4 and 2500 U/ml, with a cut-off of 0.8 U/ml for positivity. To achieve quantification of antibodies for samples exceeding the preset measuring range the automatic sample dilution was adapted according to the manufacturer's protocol.

1.4. IFNγ ELISpot analysis

For IFNγ ELISpot analysis, isolated PBMCs were thawed, rested for 2 h in AIM-V medium and then cultivated at 3.3×10^5 cells per well according to the manufactorer's instructions. In brief, cells were stimulated for 16–20 h with 2 μg/ml PepTivator® SARS-CoV-2 Prot_S Complete (Miltenyi Biotech, Bergisch Gladbach, Germany) or PMA/Ionomycin as a positive control. Bound IFNγ was visualized using a secondary anti-IFNγ antibody directly conjugated with alkaline phosphatase (1:250; ELISpotPro kit, Mabtech) followed by incubation with a 5-bromo-4-chloro-3′-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) substrate (ELISpotPro kit, Mabtech). Plates were scanned using an AID Classic Robot ELISPOT Reader and analyzed by AID ELISPOT 7.0 software (AID Autoimmun Diagnostika).

1.5. SARS-CoV-2 reactive T-cell assay

Cryopreserved cells were thawed, rested for at least 2 h in AIM-V medium and then cultured in the presence of 2 μg/ml PepTivator® SARS-CoV-2 Prot_S Complete for 16–20hrs in 96-wells U-bottom plates using 2×10^6 PBMCs per well. As a positive control, Cell Stimulation Cocktail (Thermo Fisher) was used and added for the last 4hrs. Surface and intracellular staining of PBMCs was performed according to routine protocols and using appropriate combinations of antibodies for the detection of CD3, CD4, CD8, CD45RA, CD107a, CD137, CD154, CCR7, CXCR5, PD-1, IFNγ, TNFα, IL-2, Granzyme B (all Becton Dickinson, San Diego, USA), and Fixable Viability Dye (Thermo Fisher, Waltham, USA). For intracellular staining of cytokines, Golgi transport was inhibited by Protein Transport Inhibitor Cocktail (Thermo Fisher) for 4 h prior to intracellular staining. For both approaches, surface staining was performed for 20 min at 4 °C. Afterwards, samples were fixed and permeabilized using the Cytofix/Cytoperm kit according to the manufacturer's instructions (BD Biosciences). Intracellular staining was performed in Perm/Wash buffer for 30 min at 4 °C. Samples were acquired on a FACSLyric instrument (BD Biosciences) and analyzed with FlowJo software version 10.5.3 (FlowJo LLC).

1.6. Statistical analysis

All statistical analyses were performed using the SPSS program, version 27 (Chicago, IL, USA) and R, version 4.1.1 (https://www.R-project.org/). The Kolmogorov–Smirnov test was used to analyze the distribution of the variables. In case of a parametric distribution of continuous variables, data were reported as mean and SD, and we applied the two-sided Student's t-test (comparison of two groups) for comparisons. In case of a non-parametric distribution, the results were described as median and range, and we conducted the Mann–Whitney U- and the Kruskal–Wallis tests. To test for distribution of variables χ2-squared test was used. Correlation matrix calculation and visualization was done using the psych (version 2.1.9), stats (version 3.6.2) and corrplot (version 0.92) packages in R. Correlation between variables was evaluated by Spearman's rank correlation coefficient and Bonferroni corrected for multiple testing. Clustering analysis was done using the base (version 3.6.2), stats (version 3.6.2) and pheatmap (version 1.0.12) packages in R. Data was scaled and centered before analysis. Unsupervised hierarchical clustering was conducted based on Euclidian distances between each individual data point using the Ward's minimum variance method. Transformed T-cell frequencies

and clustering results were summarized in a heatmap. For visualization, graphs were made using GraphPad Prism (GraphPad Software, San Diego, USA) showing mean values and standard error of the mean.

2. Results

2.1. Study population

40 patients suffering from autoimmune diseases (AID) and 63 healthy individuals (HC) were enrolled in the study. All study participants were vaccinated with mRNA-1273 (Spikevax), with only two healthy individuals receiving BNT162b2 (Comirnaty). Clinical characteristics are described in Table 1.

2.2. Loss of uniformity of cellular immune responses to COVID-19 vaccination

In order to investigate cellular immunity to COVID-19 vaccination in detail, we performed ELISpot as well as flow cytometric analyses of PBMCs 3–4 weeks after complete vaccination to SARS-CoV-2 (Figs. S1A–C shows the induction of T-cell responses pre- and post-vaccination). In striking contrast to the greatly diminished antibody titers in patients receiving B-cell depleting therapy, we could not detect any difference in T-cell mediated immune responses using the IFNγ ELISpot assay (Fig. 1A). Using flow cytometry we tested for a potential difference in $CD4^+$ and $CD8^+$ T-cell reactivity. We, however, observed that the frequency of IFNγ producing $CD4^+$ as well as $CD8^+$ T-cells was comparable between our patient cohort and healthy individuals and correlated well with the results from ELISpot analysis (Fig. 1B&C).

Analyzing T-cell responses in greater detail, we investigated the induction of activation markers CD137 and CD154 as well as effector cytokines including TNFα, IL-2 and GzmB in addition to the IFNγ response. In regard to the activation markers, we observed that the frequency of $CD137^+$ or $CD154^+$ $CD4^+$ T-cells as well as $CD137^+$ $CD8^+$ T-cells was again comparable between our patient cohort and healthy volunteers (Fig. 1D). As expected, CD137 induction was mostly found in $CD4^+$ and $CD8^+$ effector memory cells in healthy individuals. In contrast, CD137 upregulation was highest in $CD8^+$ TEMRA cells in AID patients, although both cohorts had similar frequencies of peripheral $CD8^+$ TEMRA cells (Fig. S2). Determining production of effector cytokines, we detected a reduced frequency of $TNF\alpha^+$ $CD4^+$ T-cells in AID patients (Fig. 1E). Of note, induction of GzmB expression was significantly reduced in our patient cohort compared to HCs as well (Fig. 1F). The induction of cytotoxic granules as indicated by CD107a, however, was equally upregulated in HCs and AID patients (data not shown).

Overall, all analyzed markers of T-cell reactivity correlated very well in HCs suggesting a finely coordinated process with a uniform involvement of $CD4^+$ as well as $CD8^+$ T-cells (Fig. 1G). This process, however, seems to be disturbed in AID patients as strength of correlations decreased in this cohort. Of special interest seems to be the almost total lack of correlation of GzmB response of $CD8^+$ T-cells with other cytokines tested (Fig. 1G).

2.3. Induction of humoral and cellular immunity appear not to be connected

Next, we wanted to investigate if cellular immune responses are linked to antibody titers following vaccination and therefore analyzed patients with and without seroconversion in our cohort.

Table 1
Baseline characteristics of the study population.

Variable	HCs	AIDs	p-value
Number	63	40	
Age [years][a]	49.8 (23–77)	54 (27–76)	0.260
Female gender, n (%)	37 (58.7)	28 (70)	0.298
Body mass index [kg/m^2][a]	24 (18.1–38.4)	24.6 (16.8–39.4)	0.252
Vaccine			
mRNA-1273 (Moderna)	61 (96.8)	40 (100)	
BNT162b2 (BioNTech/Pfizer)	2 (3.2)	–	
Disease diagnosis			
Inflammatory arthritis, n (%)		6 (15)	
Systemic sclerosis, n (%)		7 (17.5)	
Multiple sclerosis, n (%)		10 (25)	
Idiopathic inflammatory myopathies, n (%)		5 (12.5)	
other connective tissue diseases, n (%)		8 (20)	
other, n (%)		4 (10)	
Disease parameters[b]			
Remission/inactive disease, n (%)		23 (82.1)	
Low disease activity, n (%)		4 (14.3)	
Moderate/high disease activity, n (%)		1 (3.6)	
Disease duration [years][a]		4 (0–35)	
B-cell depleting therapy			
Rituximab, n (%)		33 (82.5)	
Ocrelizumab, n (%)		7 (17.5)	
Days since B cell depletion[a]		69 (0–603)	
Concomitant medication			
None, n (%)		17 (42.5)	
Corticosteroids, n (%)		2 (5)	
HCQ, n (%)		5 (12.5)	
MMF, n (%)		9 (22.5)	
MTX, n (%)		2 (5)	
AZA, n (%)		1 (2.5)	
other, n (%)		4 (10)	

HC, Healthy Control; AID, Patient with Autoimmune Disease; HCQ, Hydroxychloroquine; MMF, Mycophenolate mofetil; MTX, Methotrexate; AZA, Azathioprine.
[a] Median (range).
[b] Disease activity was assessed in 28 AID patients.

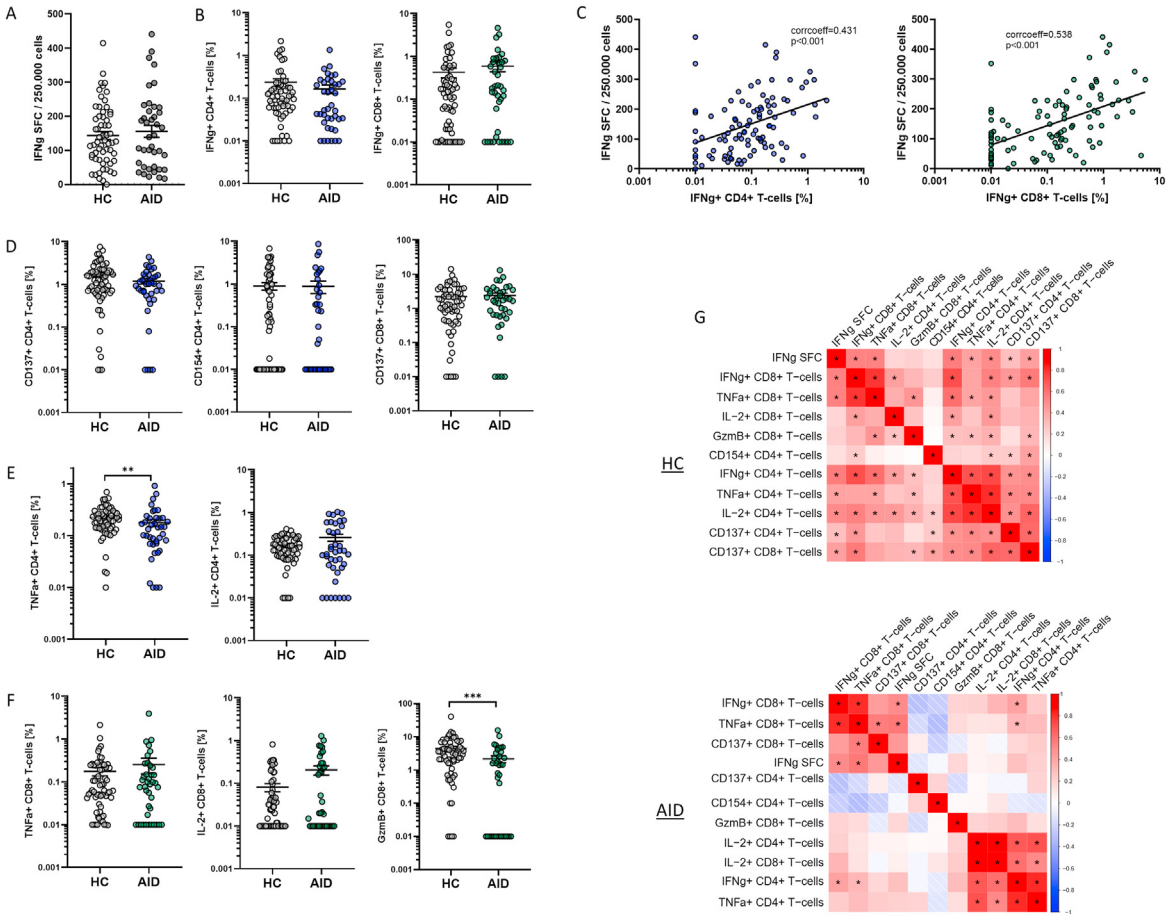

Fig. 1. Loss of uniformity of cellular immune responses in patients with B-cell depleting therapy. Graphs show (**A**) the number of IFNγ spot forming cells (SFC) in healthy controls (HC) and patients suffering from autoimmune diseases (AID) determined by ELISpot analysis; (**B**) the frequency of IFNγ-producing $CD4^+$ and $CD8^+$ T-cells in HCs and AIDs determined by flow cytometry; (**C**) correlations of IFNγ SFC numbers with the frequencies of IFNγ-producing $CD4^+$ and $CD8^+$ T-cells determined by flow cytometry; (**D**) upregulation of surface markers CD137 and CD154 on $CD4^+$ and $CD8^+$ T-cells in HCs and AIDs determined by flow cytometry; (**E&F**) the frequencies of TNFα, IL-2 and GzmB-producing $CD4^+$ and $CD8^+$ T-cells in HCs and AIDs determined by flow cytometry. All assays were performed following stimulation with a peptide pool covering the spike protein. (**G**) Correlation matrices of effector cytokine and activation marker expression of $CD4^+$ and $CD8^+$ T-cells in HCs and AIDs. (A-B, D-F) Mann-Whitney-U test; (C, G) Spearman's rank correlation; *p < 0.05; **p < 0.01; ***p < 0.001.

We noted that AID patients with detectable antibody titers tended to have higher levels of IFNγ spot-forming cells (SFCs) according to ELISpot analysis (Fig. 2A). Our flow cytometric data on IFNγ production, however, could not confirm these results and showed equal levels of $IFN\gamma^+$ $CD4^+$ and $CD8^+$ T-cells in patients and controls (Fig. 2B). Induction of surface marker CD137 was enhanced in $CD4^+$ T-cells of patients with seroconversion (Fig. 2C). The frequency of $TNF\alpha^+$ $CD4^+$ T-cells tended to be enhanced in patients with seroconversion, whereas levels of IL-2 producing $CD4^+$ T-cells were comparable (Fig. 2D). Analyzing $CD8^+$ T-cells, we found that the frequency of all tested effector molecules was not affected by the seroconversion status of our AID patients (Fig. 2E). Taken together, these data suggest that cellular immunity is only mildly affected by B-cell depleting therapy or the presence of B-cells and that the underlying disease might be responsible for the observed dysregulation of cellular immunity in our AID cohort.

2.4. Immune cell composition correlates with facets of cellular immunity

Previously, we reported that humoral immune responses in this cohort were highly affected by the number of circulating naïve B-cells [15]. Therefore, we analyzed if the absolute number of naïve or total B-cells as well as the time since the last dose of B-cell-depleting therapy correlated with cellular immune responses described above. Interestingly, the only connection we detected was a trend in correlation of total B-cell numbers with the frequency of $CD137^+$ $CD4^+$ T-cells in AID patients ($corr_{coeff} = 0.312$; $p = 0.056$, data not shown) that is in line with the increased seroconversion in these patients. Of note, in healthy individuals the number of total B-cells as well as naïve B-cells correlated negatively with $GzmB^+$ $CD8^+$ T-cells (Fig. 3A). To consolidate our data on this topic we performed hierarchical clustering based on Euclidian distances on our healthy and AID patient cohorts and defined clusters with overall good (HC2, AID2) or low (HC1, AID1) T-cell reactivity according to ELISpot and flow cytometric analyses (Fig. S3A). Neither age and sex nor body mass index helped to explain the distribution of individuals among the clusters (Figs. S3B–D). B-cell composition, on the other hand, was markedly altered in the HC clusters with an enhanced frequency of naïve and diminished frequencies of IgD + memory, IgD-memory and CD21- B-cells in HC1 (Fig. S3E). Anti-spike antibody titers were similar in HC cluster 1 and 2 (Fig. S3F). In our AID cohort, B-cell composition was no discriminating factor due to B-cell depleting therapy and thus the lack/low number of B-cells in most patients.

To complement the investigation on the effect of the immune composition on cellular immunity following vaccination, we also analyzed absolute numbers of total $CD3^+$, $CD4^+$ and $CD8^+$, naïve

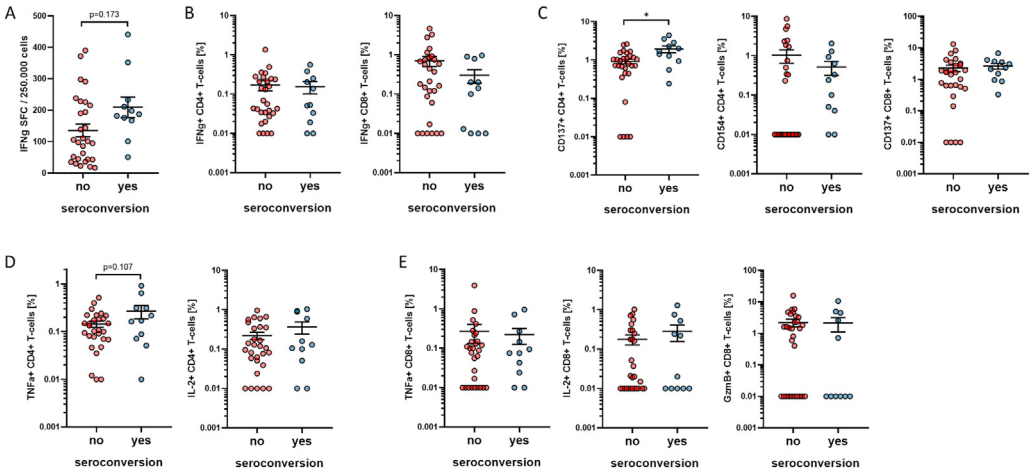

Fig. 2. Cellular immunity was mostly comparable in AID patients with and without seroconversion. Graphs show (**A**) the number of IFNγ SFCs; (**B**) the frequency of IFNγ-producing $CD4^+$ and $CD8^+$ T-cells; (**C**) upregulation of surface markers CD137 and CD154 on $CD4^+$ and $CD8^+$ T-cells; (**D&E**) the frequencies of TNFα, IL-2 and GzmB-producing $CD4^+$ and $CD8^+$ T-cells in AID patients with and without seroconversion following vaccination. (all) Mann-Whitney-U test; *p < 0.05; **p < 0.01; ***p < 0.001.

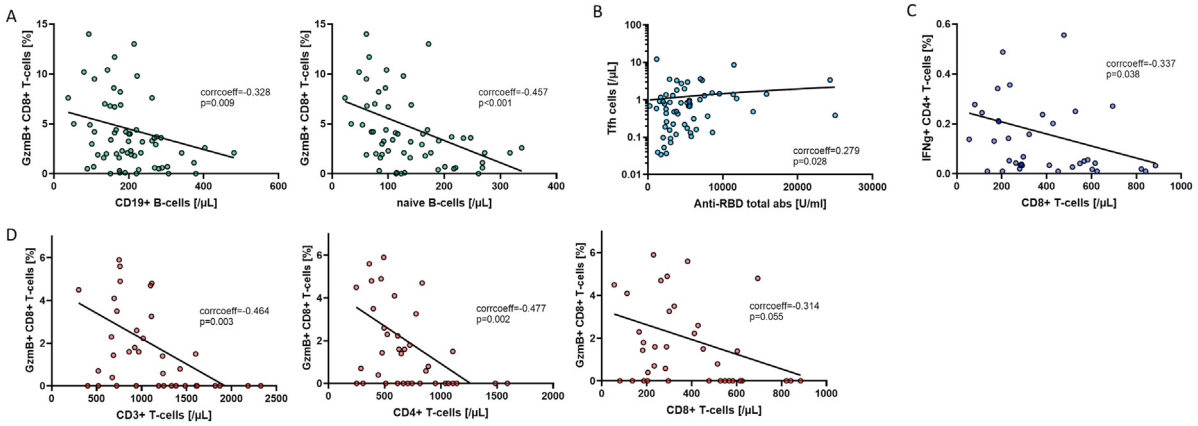

Fig. 3. Correlation of B-cell numbers with cytotoxic T-cell activity. Graphs show correlations of (**A**) the frequency of GzmB-producing $CD8^+$ T-cell with absolute numbers of total and naïve B-cells in HCs; (**B**) the total number of circulating Tfh cells with anti-RBD antibody titers in HCs; (**C**) the frequency of IFNγ-producing $CD4^+$ T-cell with absolute numbers of $CD8^+$ T-cells in AIDs; (**D**) the frequency of GzmB-producing $CD8^+$ T-cell with absolute numbers of total as well as $CD4^+$ and $CD8^+$ T-cells in AIDs. (all) Spearman's rank correlation.

$CD4^+$ and $CD8^+$ T-cells as well as T follicular helper (Tfh) cells. In HCs, we observed that the number of circulating Tfh cells correlated with anti-spike RBD antibody titer and the frequency of $CD154^+$ $CD4^+$ T-cells (Fig. 3B). This effect was not detected in the AID cohort. In our AID cohort, we observed that the frequency of IFNγ producing $CD4^+$ T-cells correlated negatively with the number of total CD8 T-cells (Fig. 3C). Furthermore, the frequency of GzmB producing $CD8^+$ T-cells correlated negatively with numbers of $CD3^+$ T-cells in general and more specifically with total and naïve $CD4^+$ and total $CD8^+$ T-cells (Fig. 3D). Taking a look at our clustering analysis (Fig. S3), the frequency of various T-cell subsets could not explain distribution into the clusters and thus might not be important for the induction of cellular immunity.

2.5. Concomitant immunosuppressive therapy has only minor effects on cellular immunity

In order to better understand factors that affect T-cell immune responses, we investigated if medication as well as different disease entities included in our cohort would affect any of the studied markers. Comparing treatment with Rituximab (RTX) and Ocrelizumab (OCR), we noted that patients with OCR showed enhanced cytokine responses in $CD4^+$ T-cells, despite having comparable levels of B-cells and time since the last treatment dose (Figs. S4A–D). In regard to additional immunosuppressive treatment, we could only observe a slight reduction in IL-2 producing $CD4^+$ T-cells, especially in patients receiving hydroxychloroquine ($p = 0.073$; Figs. S5A–E). Similarly, disease entities mostly affect frequencies of IL-2^+ $CD4^+$ T-cells with patients suffering from inflammatory arthritis having the lowest levels ($p = 0.065$; Figs. S6A–E). All other markers were not strongly affected by therapy or disease. In addition, we were unable to find a connection of disease duration with parameters of cellular immunity.

2.6. $CD4^+$ T-cell reactivity correlates with the sustainability of anti-SARS-CoV-2 antibody titers

Finally, we wanted to investigate if cellular factors and T-cell reactivity affect the sustainability of anti-SARS-CoV-2 antibody titers. Therefore, we analyzed antibody titers at 3–4 weeks after secondary immunization and before booster immunization about 6 months later. In concordance with others [16], we observed a distinct decline of anti-SARS-CoV-2 spike receptor-binding domain antibody levels in our healthy cohort over time (Fig. 4A). Pre-boost

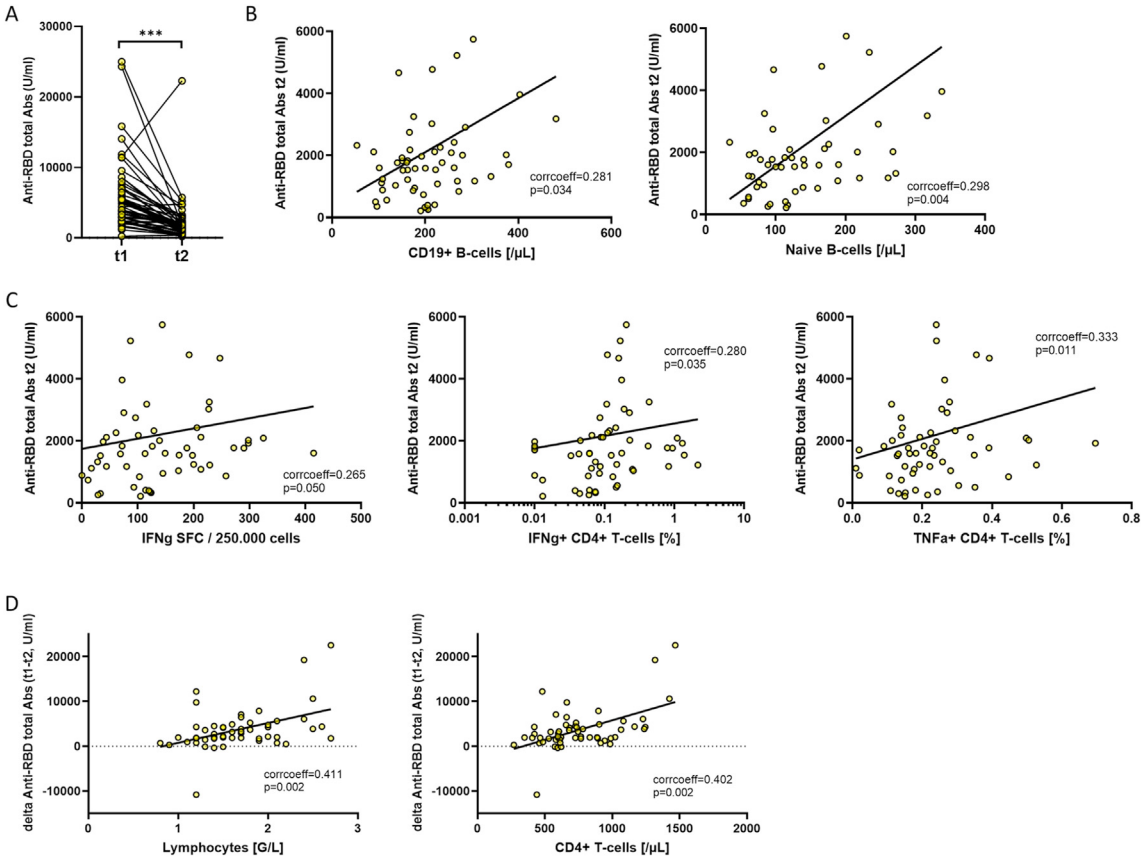

Fig. 4. The number of naïve B-cells correlates with the sustainability of anti-SARS-CoV-2 antibody titers. Graphs show (**A**) anti-RBD antibody titers of HCs at t1 (21–28 days following the second dose of vaccination) and t2 (before booster vaccination at 6 months); (**B**) correlations of anti-RBD antibody titers at t2 with absolute numbers of total and naïve B-cells in HCs; (**C**) correlations of anti-RBD antibody titers at t2 with numbers of IFNγ SFCs as well as frequencies of IFNγ- and TNFα-producing $CD4^+$ T-cells; (**D**) the change of anti-RBD antibody titers (Δ anti-RBD = t1-t2) with absolute numbers of lymphocytes and $CD4^+$ T-cells. (A) Kruskal–Wallis test; (B–D) Spearman's rank correlation; *p < 0.05; **p < 0.01; ***p < 0.001.

antibody titers, thereby, were strongly correlated with the absolute number of B-cells and even more so with the number of naïve B-cells (Fig. 4B). Of note, individuals with a higher initial reactivity in ELISpot analysis as well as spike-specific $IFN\gamma^+$ and $TNF\alpha^+$ $CD4^+$ T-cells had also higher antibody titers after 6 months (Fig. 4C). A higher absolute number of lymphocytes and more specifically $CD4^+$ T-cells at baseline was, curiously, associated with a more distinct decline of antibodies levels over time (Fig. 4D). Given the low number of seroconverted AID patients and thus limited power of information, no interpretation of the data on our patient cohort could be made.

3. Discussion

In the present work, we show that SARS-CoV-2 vaccination using mRNA vaccines elicits cellular T-cell responses in autoimmune patients under B-cell depleting therapy. Some facets of this immune response including TNFα production of $CD4^+$ T-cells and GzmB production of $CD8^+$ T-cells, however, are distinctly diminished in these patients. Consequently, it appears that the finely coordinated process of T-cell activation with a uniform involvement of $CD4^+$ as well as $CD8^+$ T-cells as seen in HCs is disturbed in AID patients. Unsurprisingly, we observed initial evidence that cellular immune responses might be affected by immune cell composition.

Humoral immunity including neutralizing antibodies is widely accepted as an important correlate for vaccine-induced protection. Accumulating evidence also points to a pivotal role for T-cells in this context, but to date cellular immunity is incompletely defined and understood [17]. Studies on patients suffering from immune-mediated inflammatory diseases [14] and multiple sclerosis [18] under B-cell depleting therapy reported induction of robust cellular immune responses using ELISpot assay analysis, despite poor antibody responses. Apostolidis et al. investigated T-cell responses in greater detail and identified augmented $CD8^+$ T-cell responses in MS patients treated with anti-CD20 antibodies and consequently suggested a compensatory $CD8^+$ T-cell activation and proliferation due to lack of antigen clearance by vaccine-induced antibodies [19]. GzmB production of $CD8^+$ T-cells in this study was comparable in HCs and patients. Our study, however, could not detect this compensatory mechanism of more vigorous $CD8^+$ T-cell responses, but rather a decrease of GzmB expression in these cells was observed. This is also in line with the finding of Graalmann et al., that described impaired CD8 response to vaccination against influenza virus in RTX-treated RA patients and in mice lacking B-cells [20]. Possible explanations for this discrepancy are the use of a more diverse patient cohort and a different time-point of cellular analysis. Of interest is also a study showing that patients suffering from primary antibody deficiency show diminished functional GzmB-producing $CD8^+$ T-cells following vaccination to SARS-CoV2, thus again suggesting a link of B-cells with cytotoxic T-cell responses. Taken together, these data suggest that vaccinating B-cell-deficient patients is still likely to provide some measure of immunity to SARS-CoV-2, especially considering that T-cells may

recognize emerging variants of concern that have escaped antibody neutralization [21].

Patients suffering from autoimmune diseases are commonly prone to abnormal T-cell reactivity with high expression of effector molecules (as reviewed in detail here [22,23]). Yet, we observed reduced effector function following vaccination at least in some aspects. These findings might simply be explained by the fact that our patient cohort received immunosuppressive treatment. The role of B-cells as antigen presenting cells and inducers of cellular immunity is well established today [24], and thus a lack of B-cells would subsequently lead to a reduction in T-cell responses. This is in line with the observed reduction of TNFα^+ CD4$^+$ T-cells and GzmB$^+$ CD8$^+$ T-cells in our patient cohort under B-cell depleting therapy. In striking contrast, our data in HCs showed elevated induction of cytotoxic CD8$^+$ T-cell response and overall cellular immune responses with lower numbers of naïve B-cells. This discrepancy clearly highlights the need for a better understanding of the cellular interplay in the induction of immune responses and how disturbances affect different arms of humoral and cellular immunity.

Certainly, an impact of underlying disease pathomechanisms cannot be excluded to also affect the cellular immune response to vaccination. In this context, it is of interest that we observed a higher percentage of peptide-induced activation of CD8$^+$ TEMRA cells in AID patients, that potentially feature immune dysfunction due to immunosenescence or exhaustion. Premature immune aging is a common feature shared by a variety of AIDs and diminished or abnormal T-cell reactivity as well as alterations in T-cell composition are hallmarks of this phenomenon [25,26]. In this regard, it is interesting to note that we observed a disconnection of T-cell responses in our AID cohort. This disconnection, moreover, seems to be driven by the T-cell composition as higher levels of IFNγ^+ CD4$^+$ T-cells were associated with lower numbers of CD8$^+$ T-cells, whereas higher levels of GzmB$^+$ CD8$^+$ T-cells were associated with lower numbers of CD4$^+$ T-cells. Immunosenescence, however, has also been recognized to impact the innate immune system [27]. Recently, it has been shown that enhanced myeloid cell response and distinct enrichment of a newly identified myeloid cell cluster after secondary immunization might be pivotal for the establishment of pronounced vaccination-induced immune responses [28]. Furthermore, Li et al. reported that the MDA5−IFNAR1 signaling pathway in antigen presenting cells is critical for the induction of CD8$^+$ T-cell responses [29]. Together, these studies highlight the importance of innate immune cells in the regulation of vaccination-induced immune responses. If and how innate cells might contribute to the effects described in this work is not clear and further studies are needed to throw light on innate, T- and B-cell interactions.

The COVID-19 pandemic has massively urged the research of cellular immune responses to vaccination with the limitation that the clinical significance is still incompletely understood, particularly amongst immunocompromised individuals. Our data advocate for a more holistic assessment of immune status in research cohorts, as several effector functions of the immune system might contribute to a full disease protection. More specifically, most studies evaluate cellular immunity using IFNγ ELISpot only and thus do not discriminate between induction of CD4 and CD8 responses and even more so do not determine other parameters that might contribute to the efficacy of vaccination such GzmB production. Broader experimental setups, including detailed T-cell evaluations, might therefore lead to better-informed vaccination strategies and will improve our understanding on the clinical importance of various aspects of cellular immunity. An accumulating number of studies follows this approach already and thus highlights the importance of multifactorial T-cell analyses following vaccination in various disease contexts [19,30,31].

Our study has some limitations: first, this is a single-center study with a limited number of patients in a heterogeneous cohort. In order to draw detailed conclusions on specific disease entities or treatment options further studies are needed. Second, we only included AID patients under B-cell depleting therapy and therefore this strategy does not allow us to dissect the effect of both factors (underlying disease and therapy) individually as discussed above. Also, some previous or concomitant medication might impact cellular immunity. The number of patients on specific treatments, however, is likely too small to robustly identify effects on T-cell reactivity. Third, we investigated only a selected set of activation markers and effector cytokines/molecules and thus other potentially interesting variations in T-cell responses could not be depicted in this study. Fourth, we merely investigated the effect of mRNA vaccination to SARS-CoV-2. Therefore, the effects of other vaccination strategies cannot be discussed.

3.1. Conclusion

In conclusion, this work suggests disturbed cellular immunity following mRNA vaccination in AID patients treated with B-cell depleting therapy. Further research is needed to better understand the interplay of immune cells in immunocompromised individuals and to determine the importance of this disturbance *in vivo*.

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Author contributions

IH, HG, MS, IS, PS and JF designed the study. IH, CS, PF, NS, MM, BD, ES, AL, BK, SH, F M−F, T S−H, BH, MK, CE, MS and JF collected clinical samples and/or data. IH, CS, PF, BD, BK and JF performed experiments. IH, CS, PF, BD, MS and JF analyzed the data and performed the statistical analysis. All authors critically reviewed and discussed the results. IH, MS and JF wrote the first draft. All authors reviewed the draft and approved the final version of the manuscript.

Availability of data and material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Medical University of Graz in April 2021 (EK 1128/2021).

Consent to participate

Written informed consent was obtained from all individual participants included in the study.

Consent for publication

The authors affirm that human research participants provided written informed consent for publication of all presented images.

Declaration of competing interest

The authors have no relevant financial or non-financial interests to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micinf.2023.105103.

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