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CORRESPONDENCE

Increased CD8 T-cell immunity after COVID-19 vaccination in lymphoid malignancy patients lacking adequate humoral response: An immune compensation mechanism?

To the Editor:

SARS-CoV-2 vaccine immunogenicity is commonly evaluated by measuring antibody titers against the SARS-CoV-2 Spike (S) protein. Previously, inferior humoral vaccination responses in patients with lymphoid malignancies have been shown.[1] This can be attributed to immune defects caused by disease or treatment. NHL and CLL treated with CD20- and MM with CD38-directed therapies, leads to long-lasting B- or plasma-cell depletion, respectively. CLL and MM are associated with hypogammaglobulinemia and aberrations in T-cell function. T-cell immunity is vital for viral clearance and long-lasting protection against COVID-19 after vaccination.[2] Moreover, high $CD8^+$ T-cells contribute to COVID-19 survival in hematological patients.[3] Studies investigating T-cell responses after vaccination in patients with lymphoid malignancies are, however, scarce and results are conflicting. This leaves a knowledge gap, underlining the urgency of an in-depth and reproducible analysis of functional SARS-CoV-2 specific T-cell responses following vaccination in hematological patients.

Patients. Adult patients diagnosed with CLL, NHL, or MM at two tertiary care centers in the Netherlands undergoing SARS-CoV-2 vaccination were included in the study. Serological and T-cell responses were evaluated pre-vaccination and 2 weeks, 3- and 6-months post-vaccination. Patients were included between March and June 2021. All participants gave written informed consent and all procedures performed were in accordance with the ethical standards of the national research committee and the 1964 Helsinki declaration (NL76863.068.21/METC 21-014).

Vaccination responses. Antibody levels were measured with an anti-SARS-CoV-2 S immunoassay (Elecsys, Roche Diagnostics International Ltd). Adequate seroconversion was defined as an Ig serum concentration ≥250 BAU/ml. $CD4^+$ and $CD8^+$ T-cell responses were evaluated by stimulation of PBMCs using a 15-mer with 11 amino acid overlapping SARS-CoV-2 spike peptide pool (SB-peptide). T-cell activation and phenotype were measured by flow cytometry: CD154, CD137, CD69, IFN-γ, TNF-α, IL-2, IL-4, PD-1, IL-17, CXCR5, and FOXP3. In parallel, SARS-CoV-2-specific CD8+ T-cells were detected using spike peptide-HLA tetramer (spike/HLA-tetramer) staining combined with differentiation markers (CD45RA and CCR7). Stimulation with CEFX peptide pool (JPT/LUMC), containing peptides of CMV and other common pathogens, was used as a positive control. A response was considered to be positive if ≥0.05% of all $CD4^+$ T-cells are spike reactive and if ≥0.005% of all $CD8^+$ T-cells are spike reactive or binding spike-specific human leukocyte antigen (HLA)-peptide tetramers. These thresholds were based on the CD4 and CD8 T-cell responses measured in nine healthy individuals (data not shown). Additional information is provided in Supplemental Methods.

Statistics. Continuous variables are described as median (IQR) and categorical variables as number and percentage of total. Antibody and T-cell responses of diagnostic subgroups were compared to population proportions using one-sample binomial testing. Missing data varied across timepoints; therefore, the number of included measurements is mentioned in the figures and confidence intervals are provided. To evaluate factors associated with humoral and cellular responses, univariable logistic regression was performed. To compare groups Chi square, Wilcoxon or Mann–Whitney *U* tests were used when applicable. All tests were two-sided with an α of 0.05. Analyses were performed using IBM SPSS Statistics (version 25.0.0.2) and Graphpad Prism (version 9.3.1).

One hundred and sixty patients diagnosed with CLL ($n = 31$), aggressive NHL ($n = 39$), indolent NHL ($n = 57$), MM ($n = 30$), and other (acute lymphoblastic leukemia $n = 2$, autoimmune pancytopenia $n = 1$) were included (Table S1). All patients received two doses of an mRNA vaccine. Eight patients with pre-vaccination positive anti-SARS-CoV-2 S Ig were excluded from the analysis.

Adequate seroconversion rates 2 weeks post-vaccination were 31% in CLL, 62% in aggressive NHL, 60% in indolent NHL, and 93% in MM patients (Figure 1A). Compared to an age-adjusted population proportion of 99%, this was significantly lower in patients with all disease categories, except MM. Recent treatment with anti-CD20 containing regimens significantly reduces adequate seroconversion rate (Figure S1A). However, when treatment was >12 months before vaccination, this seroconversion rate increased significantly to 87% (Figure S1B), indicating that sufficient B-cell recovery to elicit seroconversion takes at least 12 months after B-cell depleting therapy. Next to disease and treatment, univariable logistic regression analysis showed that only a lymphocyte count below $1*10^9$/L was a significant predictor of humoral outcome (Figure S2A), which is related to B-cell-depleting therapy. These findings are in line with previous studies.[1]

T-cell responses were measured in 49 patients lacking and 14 patients with adequate seroconversion (Table S2). Spike-specific $CD4^+$ T-cell responses significantly increased in all disease cohorts 2 weeks after mRNA vaccination, whilst control CEFX-specific T-cells

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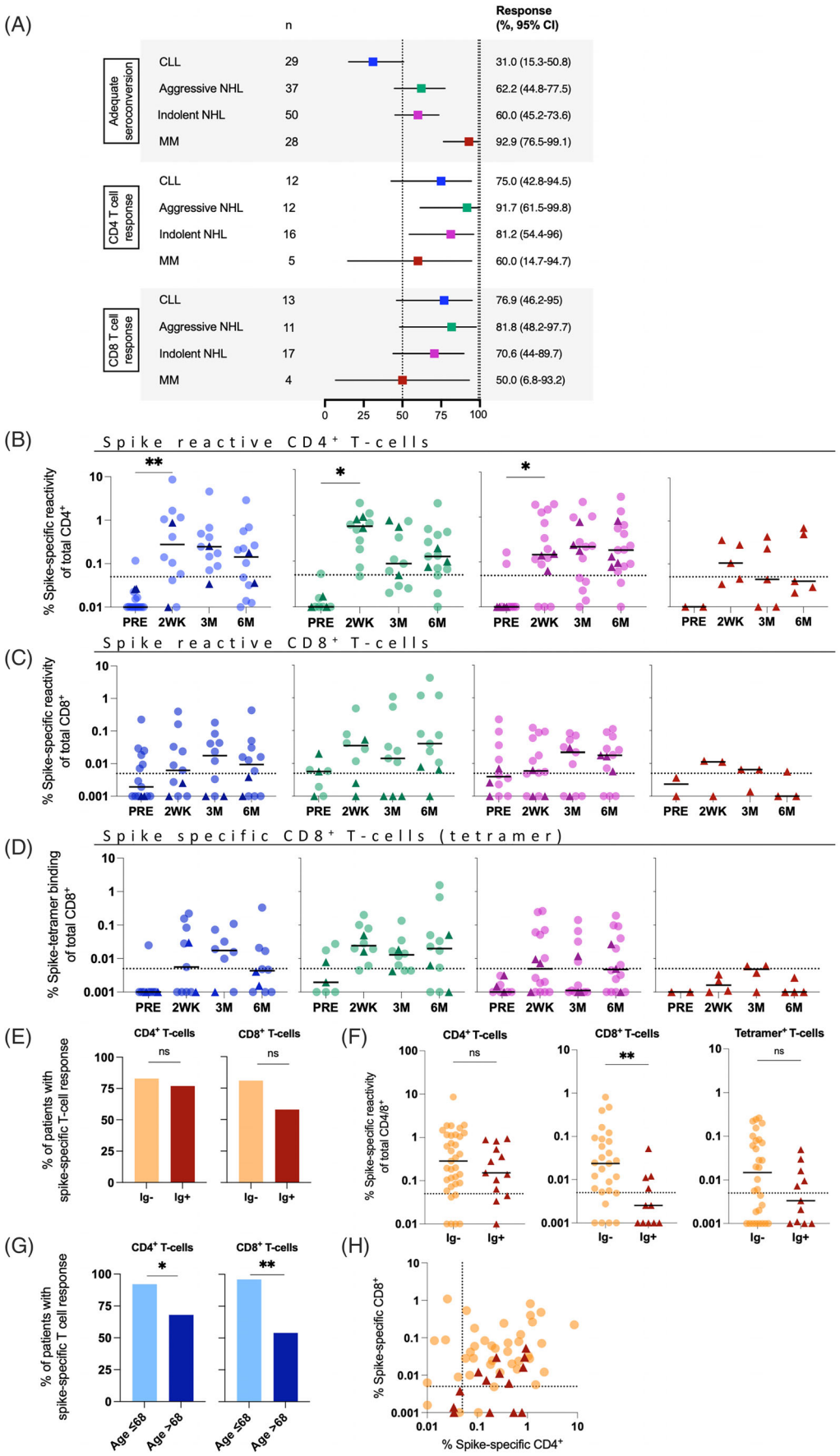

FIGURE 1 Legend on next page.

remained stable pre- and post-vaccination (Figures 1B and S3A). Spike-specific $CD8^+$ T-cell responses based on reactivation with Spike peptides (Figure 1C) and spike/HLA-tetramer staining (Figure 1D) were increased in CLL and both NHL cohorts 2 weeks after vaccination. Most spike-specific $CD4^+$ and $CD8^+$ T-cell responses remained present over time with fluctuations (Figure 1B–D) and demonstrated to exhibit a type 1 cytokine profile (Figure S4). No clear effect of a third vaccination was seen at 6 months post-initial vaccination; however, only 62% of patients received a third vaccination and this was at various time points before the 6-month time point (median: 46 days, range: 1–70). Expression of PD-1, an early T-cell activation marker, was most prominent 2 weeks after vaccination (Figure S4D,K). In the subset of Spike-specific $CD4^+$ T-cells, both T follicular helper cells and regulatory T-cells were present, though low in number (Figure S4G–H). Spike-specific $CD8^+$ T-cells were mostly effector memory phenotype (Figure S4L). These results were in line with the phenotype observed in a healthy control cohort ($n = 9$) and previous studies in healthy cohorts (data not shown).[4]

Our in-depth T-cell analysis demonstrated induction of Spike-specific $CD4^+$ responses in 75%, 92%, 81%, and 60% of CLL, aggressive NHL, indolent NHL, and MM, respectively (Figure 1A). Induction of Spike-specific $CD8^+$ responses (presence of Spike-specific and/or Spike/HLA-tetramer$^+$ $CD8^+$ T-cells) were 77%, 82%, 71%, and 50%, respectively (Figure 1A). These data collectively indicate that induction of T-cell responses does not seem to be affected by disease. However, it is important to note that, though low in number of patients analyzed, T-cell responses seem to be hampered in MM. Possible explanation is the use of dexamethasone and/or anti-CD38 therapy, which are known to negatively affect T-cells and their function.

When comparing the Spike-specific T-cell responses between patients with and without adequate seroconversion, no significant differences were observed in the percentage of patients that generated a $CD4^+$ T-cell response nor the percentage of Spike-specific $CD4^+$ T-cells in individual

FIGURE 1 Humoral and spike-specific $CD4^+$ and $CD8^+$ T-cell responses after mRNA vaccination in patients with CLL (purple), aggressive NHL (green), indolent NHL (pink) or MM (red). Response evaluation at different time points (2 weeks, 3 months, and 6 months) after initial complete vaccination, which for mRNA-1273 and BNT162b consisted of two vaccination doses, administered within a 4–5 week interval. Data for patients with CLL are shown in blue, for aggressive NHL in green, for indolent NHL in purple and, for MM in red. Anti-SARS-CoV-2 Spike antibodies were measured using anti-SARS-CoV-2 S immunoassay. Spike-specific T-cell responses were measured by thawing PBMCs and stimulating them with a spike peptide pool for 16 h, and afterwards, samples were analyzed by flow cytometry using various markers. Values were corrected for background measured in DMSO. In (B–H), each dot represents a patient; seronegative patients are depicted as circles and seropositive patients are depicted as darker triangles. (A) Response rates 2 weeks after complete vaccination, serological response rate was based on the percentage of patients with adequate anti-SARS-CoV-2 spike Ig (>250 BAU/ml). $CD4^+$ T-cell response was the percentage of patients with >0.05% of spike-specific $CD4^+$ T-cells ($CD137^+$ and/or $CD154^+$) within the total $CD4^+$ population and $CD8^+$ T-cell response was the percentage of patients with >0.005% of spike-specific CD8+ T-cells ($CD69^+$ and/or $CD137^+$ and IFN-γ^+ and/or TNF-α^+) and/or Spike/HLA-tetramer positive $CD8^+$ T-cells within the total $CD8^+$ population. All proportions were stratified based on hematological disorder and compared to expected population proportions of 99% for serological response, 100% for $CD4^+$ T-cell response, and 50% for $CD8^+$ T-cell response (shown as dotted lines in the figure) using one sample binomial testing. Clopper-Pearson method was used to estimate 95% confidence intervals. (B) Percentage of spike-specific $CD4^+$ T-cells of total $CD4^+$ T-cells measured before and after vaccination. Spike-specific $CD4^+$ T-cells were defined as $CD4^+$ T-cells expressing $CD137^+$and/or $CD154^+$ after stimulation with SARS-CoV-2 Spike peptides. Dotted line represents threshold of 0.05%. Black horizontal line represents median. Kruskal–Wallis testing does not show significant difference between hematologic disorders at 2 weeks, 3, or 6 months ($p = .526$, $p = .319$, .227). Wilcoxon test shows a significant increase in percentage of spike-specific $CD4^+$ T-cells 2 weeks after vaccination for CLL, aggressive NHL and indolent NHL ($p = .0078$, .0156, .0313) compared to pre vaccination, MM was not tested due to lack of sufficient samples. (C) Percentage of spike-specific $CD8^+$ T-cells of total $CD8^+$ T-cells measured before and after vaccination. Spike-specific $CD8^+$ T-cells were defined as $CD8^+$ T-cells having a expressing $CD69^+$and/or $CD137^+$ and IFN-γ^+ and/or TNF-α^+ after SARS-CoV2 spike peptide stimulation. Dotted line represents threshold of 0.005%. Black horizontal line represents median. Kruskal–Wallis testing does not show significant difference between hematologic disorders at 2 weeks, 3, or 6 months ($p = .369$, .921, .082). Wilcoxon test shows no significant increase in spike-specific $CD8^+$ T-cell response 2 weeks after vaccination for CLL, aggressive and indolent NHL ($p = .195$, .250, .922) compared to pre-vaccination, MM was not tested due to lack of sufficient samples. (D) Percentage of Spike/HLA-tetramer positive $CD8^+$ T-cells of total $CD8^+$ T-cells measured before and after vaccination. Dotted line represents threshold of 0.005%. Black horizontal line represents median. Kruskal–Wallis testing does not show significant difference between hematologic disorders at 2 weeks, 3, or 6 months ($p = .146$, .225, .188). Wilcoxon test shows no significant increase in tetramer positive $CD8^+$ T-cell responses 2 weeks after vaccination for CLL, aggressive and indolent NHL ($p = .063$, .063, .094) compared to pre-vaccination, MM was not tested due to lack of sufficient samples. (E) Percentage of patients with spike-specific $CD4^+$ or $CD8^+$ T-cell responses in adequately seroconverted (Ig^+) or seronegative (Ig^-) patients. Significance was tested by Chi square test ($CD4^+$ $p = .640$, $CD8^+$ $p = .143$). (F) Frequency of spike-specific $CD4^+$ or $CD8^+$ T-cells in adequately seroconverted (Ig^+) or seronegative (Ig^-) patients. Significance was tested by Mann–Whitney U tests ($CD4^+$ $p = .332$, $CD8^+$ $p = .007$, tetramer $p = .21$). (G) Percentage of patients with a spike-specific $CD4^+$ or $CD8^+$ T-cell response split by age under or above 68 years. Significance was tested by Chi square test ($CD4^+$ $p = .033$, $CD8^+$ $p = .001$). (H) Percentage of spike-specific $CD8^+$ T-cells ($CD69^+$and/or $CD137^+$ and IFN-γ^+ and/or TNF-α^+) of total $CD8^+$ T-cells (y-axis) plotted against percentage of spike-specific $CD4^+$ T-cells ($CD137^+$and/or $CD154^+$) of total $CD4^+$ T-cells (x-axis). All patients at timepoint 2 weeks or 3 months, independent of cohort, are shown in the figure and dark triangles depict patients with an adequate anti-spike antibody response. Dotted lines either represent threshold for spike-specific $CD8^+$ T-cell response on y-axis of 0.005% or spike-specific $CD4^+$ T-cell response on x-axis of 0.05%. ns, not significant; *$p < .05$; **$p < .01$ or ***$p < .001$. PRE, before vaccination; 2W, 2 weeks after complete vaccination; 3M, 3 months after complete vaccination; 6M, 6 months after complete vaccination.

patients (Figures 1E,F and S2B), indicating that the lack of humoral responses was not caused by lack of $CD4^+$ T-cell help (Figure 1A). Furthermore, no difference was observed in the percentage of patients that generated a CD8+ T-cell response (Figures 1E and S2C); however, the frequency of Spike-specific $CD8^+$ T-cells was significantly higher in patients lacking serological response (Figure 1F) 2 weeks post-vaccination, which could represent an immune compensation mechanism that might contribute to a survival advantage in case of severe COVID-19.[3]

T-cell immunity is known to decline with advanced age. In this study, age was the only significant predictor of Spike-specific T-cell responses ($CD4^+$ OR: 0.18, $CD8^+$ OR: 0.05). (Figure S2B,C), both Spike-specific $CD4^+$ and $CD8^+$ T-cell responses decline significantly for patients aged over 68 years (Figure 1G).

Though cellular responses after SARS-COV-2 vaccination in patients with lymphoid malignancies have been suggested,[5] we for the first time show with a highly specific and reproducible technique that nearly all lymphoid malignancy patients exhibit a good Spike-specific $CD4^+$ and $CD8^+$ T-cell response 2 weeks to 3 months after vaccination (Figure 1H).

Limitations of this study include a limited sample size, making it difficult to perform subgroup analysis especially within disease cohorts. Therefore, analyzing specific treatment-related effects is not possible. However, this cohort does represent a real-world situation for patients in two secondary-/tertiary-care centers in the Netherlands. In addition, the peptide pools used in the T-cell assays were based on the ancestral Wuhan strain sequence which is not the current circulating variant. However, it is important to note that it has been shown that T-cell responses induced by mRNA-1273 and BNT162b2 are minimally affected by the mutations found in omicron.[6]

In conclusion, this study demonstrates that the majority of patients lacking adequate seroconversion following SARS-CoV-2 mRNA vaccination were able to generate a cellular immune response. Moreover, a hampered humoral response is compensated by a stronger cellular response, which indicates that vaccination is of significance also in patients lacking seroconversion.

AUTHOR CONTRIBUTIONS

Lara S. Boerenkamp and Cilia R. Pothast contributed equally to this manuscript. Mirjam H. M. Heemskerk and Catharina H. M. J. Van Elssen contributed equally to this manuscript. Mirjam H. M. Heemskerk, Inge H. M. van Loo, and Catharina H. M. J. Van Elssen designed the study. Lara S. Boerenkamp collected data, performed data management, analyzed the data, made figures, and wrote the manuscript. Cilia R. Pothast designed experimental set-up, performed experiments, analyzed the data, made figures, and wrote the manuscript. Romy C. Dijkland and Kayleigh van Dijk performed experiments. Gwendolyn N. Y. van Gorkom assisted with statistical data analysis. Lotte Wieten collected samples. All authors critically revised the manuscript and reviewed and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, Lara S. Boerenkamp, upon reasonable request.

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REFERENCES

1. Jiménez M, Roldán E, Fernández-Naval C, et al. Cellular and humoral immunogenicity of the mRNA-1273 SARS-CoV-2 vaccine in patients with hematologic malignancies. *Blood Adv*. 2022;6(3):774-784.
2. Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol*. 2022;23(2):186-193.
3. Bange EM, Han NA, Wileyto P, et al. $CD8^+$ T cells contribute to survival in patients with COVID-19 and hematologic cancer. *Nat Med*. 2021;27(7):1280-1289.
4. Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses. *Nature*. 2020;586(7830):594-599.

5. Marasco V, Carniti C, Guidetti A, et al. T-cell immune response after mRNA SARS-CoV-2 vaccines is frequently detected also in the absence of seroconversion in patients with lymphoid malignancies. *Br J Haematol*. 2022;196(3):548-558.
6. Tarke A, Coelho CH, Zhang Z, et al. SARS-CoV-2 vaccination induces immunological T cell memory able to cross-recognize variants from Alpha to Omicron. *Cell*. 2022;185:847-859.e11.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.