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Letter

Variable cellular responses to SARS-CoV-2 in fully vaccinated patients with multiple myeloma

Adolfo Aleman,^{1,2,3,15} Bhaskar Upadhyaya,^{2,3,15} Kevin Tuballes,⁴ Katerina Kappes,^{2,3} Charles.R. Gleason,^{5,6} Katherine Beach,^{5,6} Sarita Agte,^{2,3} Komal Srivastava,^{5,6} PVI/Seronet Study Group, Oliver Van Oekelen,^{1,2,3} Vanessa Barcessat,⁴ Nina Bhardwaj,^{2,3,4,7} Seunghee Kim-Schulze,^{4,8,9} Sacha Gnjatic,^{2,3,4,6,8,9} Brian Brown,^{4,8,10} Carlos Cordon-Cardo,^{6,9,10} Florian Kramer,^{5,6} Miriam Merad,^{2,3,4,8,9} Sundar Jagannath,^{2,3} Ania Wajnberg,^{11,12} Viviana Simon,^{5,6,13,14,*} and Samir Parekh^{2,3,4,9,*}

¹Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

²Department of Medicine, Hematology and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

³Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁴Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁵Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁶Department of Pathology, Molecular and Cell-based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁷Department of Urology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁸Human Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA

⁹Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹⁰Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, NY, USA

¹¹Department of Internal Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹²Department of Geriatrics and Palliative Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹³Division of Infectious Disease, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹⁴Global Health and Emerging Pathogen Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

¹⁵These authors contributed equally

*Correspondence: viviana.simon@mssm.edu (V.S.), samir.parekh@mssm.edu (S.P.)

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines have been proven to be highly effective at preventing severe disease and mortality in healthy individuals; however, immunocompromised individuals with hematologic malignancies are at increased risk of severe COVID-19 manifestations (Bakouny et al., 2020; Mulligan et al., 2020). To date, most studies assessing immune responses to SARS-CoV-2 vaccines in patients with multiple myeloma (MM) have primarily focused on serological analysis (Stampfer et al., 2021; Van Oekelen et al., 2021). We recently reported that patients with MM mount highly variable anti-SARS-CoV-2 Spike (anti-S) IgG antibody responses after two doses of SARS-CoV-2 vaccines, with a complete absence of antibody responses in 15% of those patients (Van Oekelen et al., 2021).

T cell responses to SARS-CoV-2 have been detected in 41%–88% of COVID-19 convalescent individuals who have undetectable anti-S IgG antibodies (Sekine et al., 2020; Steiner et al., 2021). In immunocompromised patients without hematologic malignancies, SARS-CoV-2 vaccines elicit T cell responses even in patients without anti-S IgG antibodies (Apostolidis et al., 2021). The production of SARS-CoV-2 T cell immunity is, how-

ever, much lower in patients with hematologic malignancies that require steroid use (Ehmsen et al., 2021).

We wanted to determine whether MM patients without detectable anti-S IgG antibodies to SARS-CoV-2 immunization (seronegative) had detectable SARS-CoV-2 B and T cell responses after SARS-CoV-2 vaccination, which would possibly provide some protection against severe disease even in the absence of anti-S antibodies. In order to assay quantitative and qualitative differences in T cell responses, we adopted a high-resolution flow cytometry assay that incorporates multiple cytokines and activation markers. Such data are urgently required to guide masking, social distancing, and passive antibody/booster vaccination strategies for potentially vulnerable MM patients treated with these anti-cancer agents as we enter the second fall season of the COVID-19 pandemic.

B and T cell responses were profiled in 44 patients with MM (17 seronegative and 27 seropositive) and 12 healthy participants at least two weeks after their second mRNA SARS-CoV-2 vaccine dose (BNT162b2 Pfizer-BioNTech, n = 42; mRNA-1273 Moderna, n = 14). The clinical characteristics are presented in Table S1. SARS-CoV-2-specific IgG antibodies

were measured in these cohorts through the use of the COVID-SeroKliar Kantaro SARS-CoV-2 IgG test (Figure S1A). The majority (76%, 13/17) of seronegative MM patients were either on anti-BCMA or anti-CD38 therapies (Figure S1B). Patients with MM were all receiving care at the Icahn School of Medicine at Mount Sinai, New York.

Because SARS-CoV-2 vaccination failed to induce anti-S antibody response in seronegative MM patients, we first investigated whether this lack of antibody response reflected on the inability of the SARS-CoV-2 vaccines to induce spike-reactive B cells; our investigation used flow cytometry in peripheral blood mononuclear cells (PBMC) of vaccinated MM patients and healthy individuals. Although spike-protein-reactive B cells were detected in all but one seropositive MM patient (24/25, 96%) as well as in all of the healthy individuals, only 40% (6/15) of the seronegative MM patients harbored spike-protein-reactive B cells (Figure S1C). Seronegative patients also had lower B cell numbers in their peripheral blood compared to the seropositive MM group (p < 0.0015, Figure S1D). There was a direct correlation between the presence of spike-reactive B cells and anti-S IgG antibody concentration



(spearman $r = 0.44$, $p = 0.002$) as well as a direct correlation between absolute B cell count and anti-S IgG antibody concentration (spearman $r = 0.51$, $p = 0.00047$). In addition to the diminished absolute B cell counts, we also observed significantly reduced total CD4⁺ T cell counts in the seronegative MM patients compared to the seropositive ones ($p = 0.0065$, Figure S1E). No other significant differences in total white blood cell, lymphocyte, neutrophil, monocyte, or total CD8⁺ T cell counts were seen among the groups.

It has been posited that even patients with undetectable anti-S antibodies after vaccination may mount T cell protection from severe disease. To comprehensively screen for T cell responses, we stimulated PBMC with predicted SARS-CoV-2 HLA class I and II directed peptide pools and measured IFN- γ , TNF- α , IL-2, and GM-CSF simultaneously within CD4⁺ and CD8⁺ T cells through the use of intracellular cytokine staining (ICS)-Flow after 6 h of stimulation. Selected SARS-CoV-2 HLA class I and II peptides were 8-15-mers and 15-mers, respectively, and have been previously shown to stimulate SARS-CoV-2-specific T cells in COVID-19 convalescent patients (Grifoni et al., 2020). Activated CD4⁺ cells were identified using activation markers CD154 and CD69 (Figure S1F). Activated CD8⁺ T cells were identified using degranulation marker CD107a and activation marker CD69. Seropositive MM patients had IFN- γ , TNF- α , IL-2, or GM-CSF-expressing CD4⁺ T cells at similar levels to those found in age-matched healthy controls. In contrast, seronegative MM patients had significantly reduced CD4⁺ T cell responses compared to those of healthy controls and seropositive MM patients ($p < 0.005$, Figures S1G–S1J). There was a significant correlation between SARS-CoV-2 CD4⁺ T cell responses and anti-S IgG antibody concentration across the cohort ($r = 0.56$, $p < 0.001$, Figure S1K). We observed that 96% of the seropositive MM patients (25/26) had a CD4⁺ T cell response, similar to that of healthy controls (Figure S1L). In contrast, only 35% (6/17) of the seronegative MM patients had a CD4⁺ T cell response (Figure S1L). The percentage of patients with CD8⁺ T cell responses was not significantly different among cohorts; 50% of the healthy controls (6/12) and seropositive MM patients (12/24) mounted a CD8⁺

T cell response, compared to 28% of seronegative MM patients (4/14). Fewer patients on active anti-BCMA bispecific therapy (2/6, 33%) or anti-CD38 antibody therapy (13/19, 68%) mounted SARS-CoV-2-specific CD4⁺ T cell responses (Figure S1M) compared to patients on other myeloma therapeutics (9/10, 90%) or anti-BCMA CAR-T (8/9, 89%).

T cells co-expressing multiple cytokines (termed “polyfunctional”) are known to be induced in viral infections, and they provide protective immunity (Duvall et al., 2008). Distributions of cytokine-expressing monofunctional and polyfunctional CD4⁺ T cells were very similar between seropositive and healthy controls, and IL-2 and IFN- γ -cytokine-alone-expressing CD4⁺ T cells were the dominant fraction within the total cytokine response. However, the seronegative MM patients showed a dominant IL-2 monofunctional T cell response. CD8⁺ T cell cytokine response was largely monofunctional across all of our cohorts, with IFN- γ -expressing CD8⁺ T cells dominating the phenotypic makeup of the cytokine response.

ICS-Flow-measured SARS-CoV-2-specific T cell responses were confirmed through the use of ELISpot assays in a subset of patients. All healthy controls and MM seropositive patients produced IFN- γ ⁺ spot-forming cells (SFC) in response to CD4 peptide pool stimulation, and the medians were 58 and 73 spots per 400,000 plated PBMCs respectively; this was an order of magnitude higher than the responses of seronegative MM patients, which only showed a median of two spots per 400,000 PBMCs ($p < 0.001$). Stimulation with CD8 peptide pool resulted in lower production of IFN- γ ⁺ SFC throughout our cohort and was not significantly different across MM patient groups and healthy individuals (median 14 IFN- γ ⁺ spots in healthy individuals, median 19 IFN- γ ⁺ spots in seropositive versus 1 in seronegative MM patients, per 400,000 plated PBMCs).

Our findings indicate a high degree of variability in SARS-CoV-2-specific B and T cell responses in patients with MM. The unexpected lack of T cell responses, coupled with an absence of anti-S antibodies following SARS-CoV-2 vaccination, particularly in MM patients actively receiving anti-CD38 and anti-BCMA antibody-based therapies, is of concern,

and it emphasizes the need for serological testing after vaccination to identify this specific subgroup of MM patients. With the current rapid spread of more transmissible viral variant (e.g., Delta variant), booster vaccination, continuing safety precautions, and passive antibody treatments should be considered in order to prevent morbidity and mortality from COVID-19 in MM patients with suboptimal vaccine responses.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.ccell.2021.09.015>.

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AUTHOR CONTRIBUTIONS

V.S., A.W., S.P., and PVI/Seronet Study Group provided conceptualization, methodology, analysis, and resources for this work. A.A., K.K., K.B., K.S., S.A., C.R.G., and PVI/Seronet Study Group were involved in organizational aspects of the clinical studies, patient recruitment, data collection, and analysis. A.A., O.V.O., S.A., C.R.G., C.C.C., and F.K. were involved in design, data collection, analysis, visualization, and interpretation of serological data. A.A., B.U., K.T., V.B., S.G., and S.K.S. were involved in design, execution, analysis, visualization, and interpretation of T and B cell assays. S.J., A.W., S.P., and V.S. were involved in different aspects of patient care. B.U., N.B., S.G., B.B., M.M., S.J., A.W., V.S., and S.P. provided interpretation of the data and

conceptualization of the first manuscript draft. A.A., B.U., A.W., V.S., and S.P. contributed to the writing of the first manuscript draft. All coauthors provided critical edits to the initial manuscript draft and approved the final version.

DECLARATION OF INTERESTS

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Viviana Simon and Carlos Cardo-Cordon are listed on the serological assay patent application as co-inventors. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Florian Krammer has consulted for Merck and Pfizer (before 2020) and is currently consulting for Seqirus and Avimex. The Krammer laboratory is collaborating with Pfizer on animal models of SARS-CoV-2. Sundar Jagannath reports consulting fees from Bristol Myers Squibb (Celgene), Janssen, Karyopharm Therapeutics, Merck, Sanofi, and Takeda Pharmaceuticals. Samir Parekh reports consulting fees from Foundation Medicine. Sacha Gnjatic reports past consultancy and/or advisory roles for Merck and OncoMed and past or current research funding from Bristol-Myers Squibb, Genentech, Boehringer-Ingelheim, Pfizer, Takeda, and Regeneron. Nina Bhardwaj reports consultancy and/or advisory roles for Novartis, Apricity, Rome Therapeutics, CureVac, Genotwin, BioNTech, Gildea, Tempest Therapeutics, Boehringer Ingelheim, and Rubis Therapeutics. Other authors report no relevant conflicts of interest.

REFERENCES

- Apostolidis, S.A., Kakara, M., Painter, M.M., Goel, R.R., Mathew, D., Lenzi, K., Rezk, A., Patterson, K.R., Espinoza, D.A., Kadri, J.C., et al. (2021). Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. *Nat. Med.* <https://doi.org/10.1038/s41591-021-01507-2>.
- Bakouny, Z., Hawley, J.E., Choueiri, T.K., Peters, S., Rini, B.I., Warner, J.L., and Painter, C.A. (2020). COVID-19 and Cancer: Current Challenges and Perspectives. *Cancer Cell* 38, 629–646. <https://doi.org/10.1016/j.ccell.2020.09.018>.
- Duvall, M.G., Precopio, M.L., Ambrozak, D.A., Jaye, A., McMichael, A.J., Whittle, H.C., Roederer, M., Rowland-Jones, S.L., and Koup, R.A. (2008). Polyfunctional T cell responses are a hallmark of HIV-2 infection. *Eur. J. Immunol.* 38, 350–363. <https://doi.org/10.1002/eji.200737768>.
- Ehmsen, S., Asmussen, A., Jeppesen, S.S., Nilsson, A.C., Østerlev, S., Vestergaard, H., Justesen, U.S., Johansen, I.S., Frederiksen, H., and Ditzel, H.J. (2021). Antibody and T cell immune responses following mRNA COVID-19 vaccination in patients with cancer. *Cancer Cell* 39, 1034–1036. <https://doi.org/10.1016/j.ccell.2021.07.016>.
- Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al. (2020). Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell* 181, 1489–1501.e15. <https://doi.org/10.1016/j.cell.2020.05.015>.
- Mulligan, M.J., Lyke, K.E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Raabe, V., Bailey, R., Swanson, K.A., et al. (2020). Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 586, 589–593. <https://doi.org/10.1038/s41586-020-2639-4>.
- Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Strålin, K., Gorin, J.B., Olsson, A., Llewellyn-Lacey, S., Kamal, H., Bogdanovic, G., Muschiol, S., et al.; Karolinska COVID-19 Study Group (2020). Robust T Cell Immunity in Convalescent Individuals with Asymptomatic or Mild COVID-19. *Cell* 183, 158–168.e14. <https://doi.org/10.1016/j.cell.2020.08.017>.
- Stampfer, S.D., Goldwater, M.S., Jew, S., Bujarski, S., Regidor, B., Daniely, D., Chen, H., Xu, N., Li, M., Green, T., et al. (2021). Response to mRNA vaccination for COVID-19 among patients with multiple myeloma. *Leukemia*. <https://doi.org/10.1038/s41375-021-01354-7>.
- Steiner, S., Schwarz, T., Corman, V.M., Sotzny, F., Bauer, S., Drosten, C., Volk, H.D., Scheibenbogen, C., and Hanitsch, L.G. (2021). Reactive T Cells in Convalescent COVID-19 Patients With Negative SARS-CoV-2 Antibody Serology. *Front. Immunol.* 12, 687449. <https://doi.org/10.3389/fimmu.2021.687449>.
- Van Oekelen, O., Gleason, C.R., Agte, S., Srivastava, K., Beach, K.F., Aleman, A., Kappes, K., Mouhieddine, T.H., Wang, B., Chari, A., et al.; PVI/Seronet team (2021). Highly variable SARS-CoV-2 spike antibody responses to two doses of COVID-19 RNA vaccination in patients with multiple myeloma. *Cancer Cell* 39, 1028–1030. <https://doi.org/10.1016/j.ccell.2021.06.014>.