Severe Acute Respiratory Syndrome Coronavirus 2 Third Vaccine Immune Response in Multiple Sclerosis Patients Treated with Ocrelizumab

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The introduction of a third-dose vaccination along with new variants of concern raises questions regarding serology and T-cell responses in patients with multiple sclerosis (pwMS) treated with B-cell depletion who develop attenuated humoral response to vaccines. The aim of this study was to longitudinally evaluate humoral and cellular response to SARS-CoV-2 mRNA vaccine in ocrelizumab-treated pwMS before and following a third vaccine dose. Following the third vaccine dose, patients who are low or nonresponders following initial vaccination did not increase antibody titers. In healthy controls and ocrelizumab-treated pwMS, cellular response decreased 6 months after initial vaccination and increased significantly after the third dose.

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Concerns of waning immunity together with the resurgence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), despite a high percentage of the population being vaccinated with 2 doses of the mRNA coronavirus disease 2019 (COVID-19) vaccine, led to the recommendation of a third vaccine dose. Patients with multiple sclerosis (pwMS) treated with B-cell depletion treatments such as ocrelizumab (OCR) are able to generate a robust T-cell response following 2 doses of the mRNA vaccine.[1–3] However, an attenuated humoral response was observed.[1,4,5] The cellular response to SARS-CoV-2 is recognizing multiple sites of the receptor-binding domain (RBD) region, thus offering broad reactivity against spike epitopes. Although SARS-CoV-2 variants of concern (VOCs) are able to evade infection- or vaccination-induced neutralizing antibodies,[6] a robust T-cell response against VOCs, including the newest Omicron variant, was recently described.[7,8] Preservation of vaccine-induced T-cell responses may offer some level of protection to individuals with low antibody response. Whether OCR will have an impact on the longevity of the anti–SARS-CoV-2 response, and the effect of a third dose on the immune response of patients treated with OCR, are unknown. We assessed longitudinal humoral and T-cell response in healthy controls (HCs) and pwMS treated with OCR before and after a third BNT162b2 dose. This information can help guide treatment and vaccination recommendations.

Subjects and Methods

Participants and Setting

This single-center study was performed at Hadassah Medical Center, Jerusalem, Israel. Participants were vaccinated between December 2020 and October 2021 and donated blood for antibody and T-cell assessments 2 to 8 weeks and 6 months after their second dose and 2 to 8 weeks after their third vaccine dose (BNT162b2; Pfizer [New York, NY]/BioNTech [Mainz, Germany]). None of the participants was infected with COVID-19 prior to December 31, 2021. All participants provided written informed consent (975-20 Hadassah Medical Organization).

SARS-CoV-2 Immunoglobulin G

Serology response was measured using spike RBD Architect SARS-CoV-2 immunoglobulin G (IgG) II Quant assay (Abbott Diagnostics, Illinois, US). Positive and borderline response defined by IgG titer were ≥50 and 50–100 arbitrary units (AU)/ml, respectively.

Interferon-γ Enzyme-Linked Immunosorbent Spot and Cytokine Analysis

SARS-CoV-2–specific T-cell response was assessed using T-SPOT Discovery SARS-CoV-2 (Oxford Immunotec), using freshly isolated

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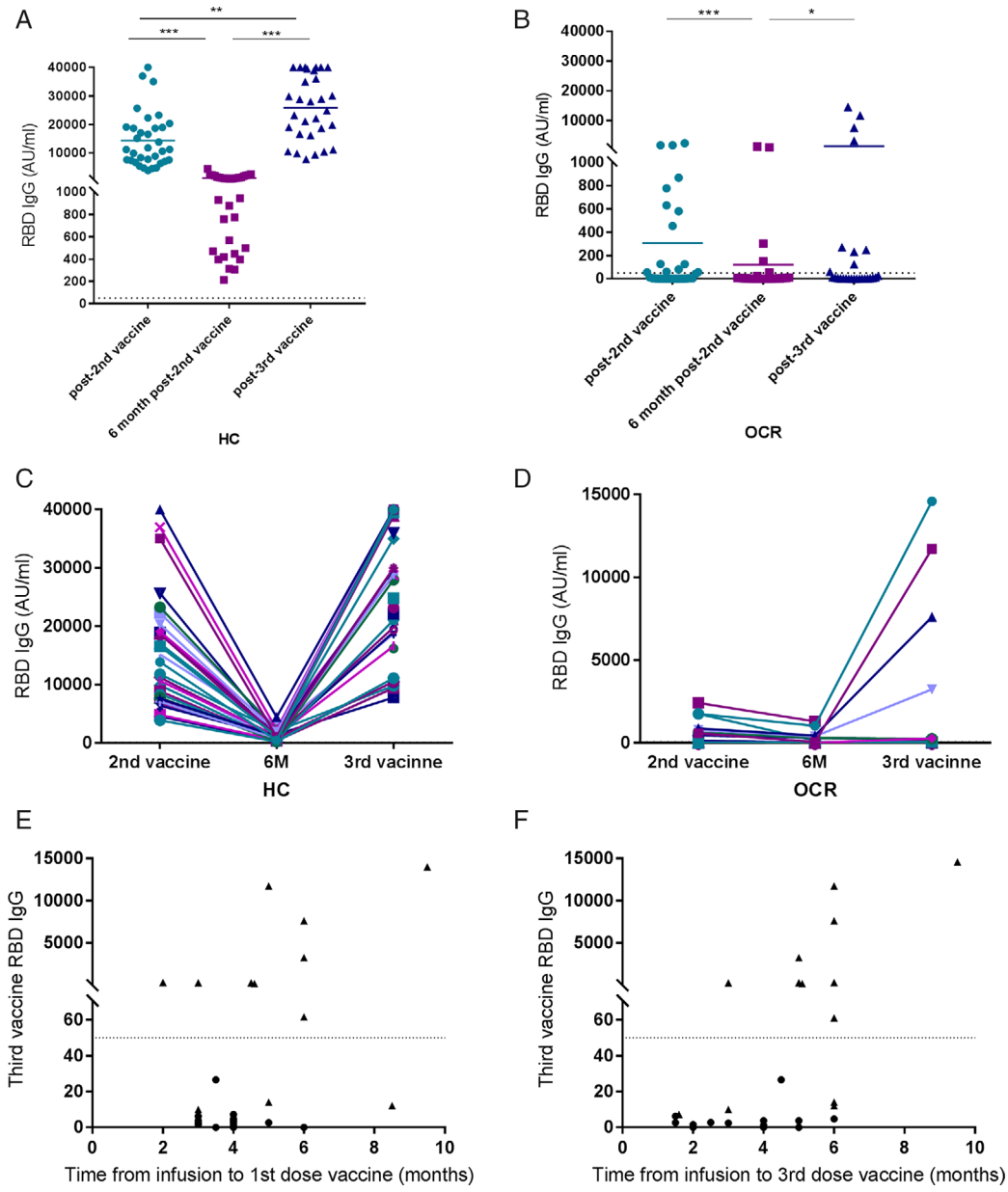

FIGURE 1: Serology response to the third dose of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccine in patients with multiple sclerosis (MS) treated with ocrelizumab (OCR) and healthy controls (HCs). (A–D) Longitudinal SARS-CoV-2 receptor-binding domain (RBD) immunoglobulin G (IgG) titers in HCs and MS patients treated with OCR at 3 time points. (A) HCs 2 to 4 weeks after the second vaccine dose: $n = 34$, 14,352 ± 9,453 arbitrary units (AU)/ml; 6 months after the second vaccine: $n = 34$, 1,241 ± 885 AU/ml; and 2 to 8 weeks after the third vaccine dose: $n = 30$, 25,851 ± 11,302 AU/ml. (B) MS patients treated with OCR: 2 to 4 weeks after the second vaccine dose: $n = 32$, 307.6 ± 604.2 AU/ml; 6 months after the second vaccine dose: $n = 24$, 121.2 ± 329.2 AU/ml; and 2 to 8 weeks after the third vaccine dose: $n = 27$, 1,413 ± 3,742 AU/ml. (C) RBD-IgG titers in 32 HCs. Each line represents one participant. (D) RBD-IgG titers in 32 patients with MS treated with OCR. Each line represents one participant. (E, F) Correlation between time from OCR infusion to vaccination. Triangular represent seropositivity after the second vaccine dose. The dotted lines indicate the cutoff for a positive response (≥50 AU/ml). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Data are presented as mean ± standard deviation. [Color figure can be viewed at www.annalsofneurology.org]

peripheral blood mononuclear cells.[1] Results are presented as the number of interferon-γ (IFNγ) spot-forming cells (SFCs/250,000 cells). Positive response is defined as SFC ≥ 6. Interleukin (IL)-2, IL-4, IL-6, IL-10, tumor necrosis factor α (TNFα), and IL-17 levels were measured in supernatants using a BD (Franklin Lakes, NJ) Cytometric Bead Array Human Inflammatory Cytokines Kit.

Statistical Analysis

Statistical analyses were performed using 1-way analysis of variance, Student *t* test, and Pearson correlation coefficient. The results are presented as mean (standard deviation [SD]). Two-sided *p* values are statistically significant at ≤0.05.

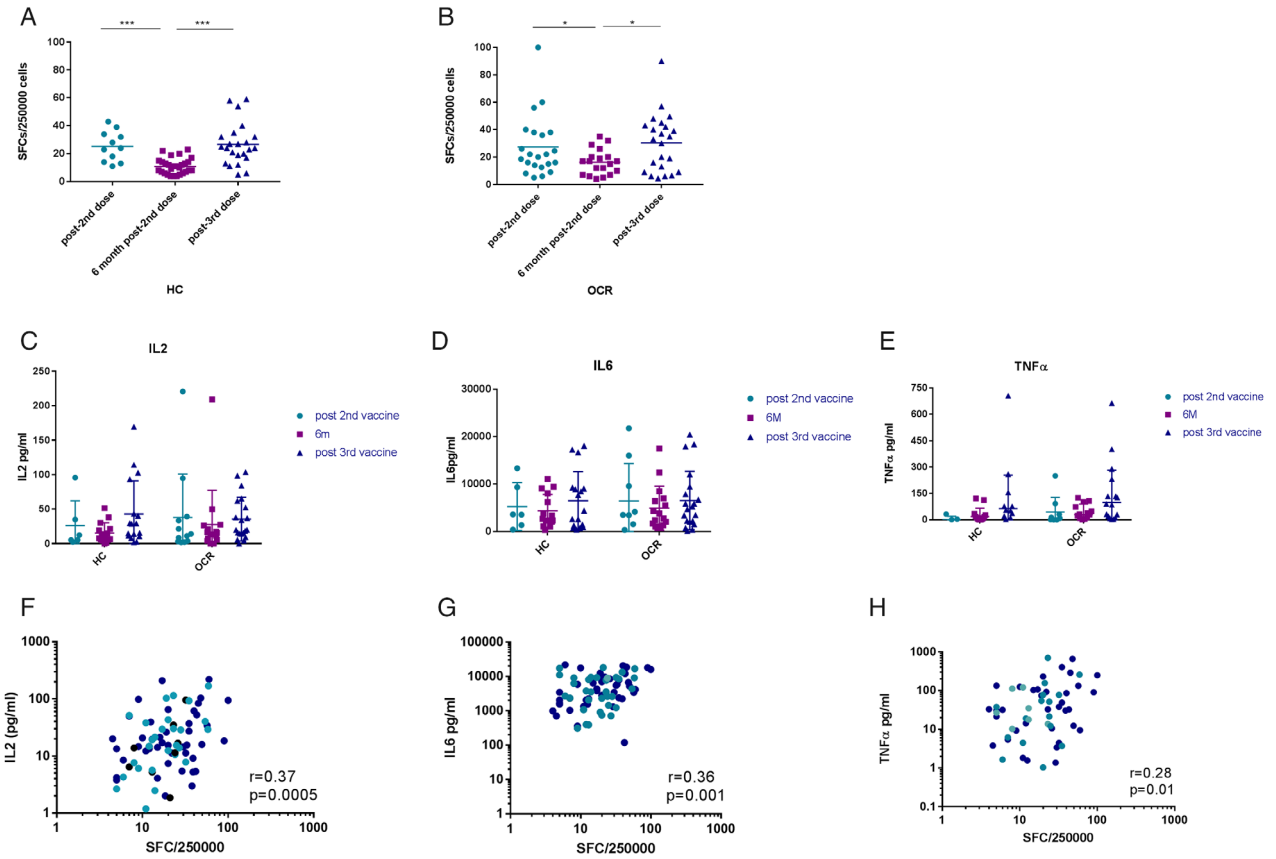

FIGURE 2: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike-specific T-cell response following vaccination with 3 vaccine doses.(A-D) Longitudinal postvaccination T-cell responses to SARS-CoV-2 peptides. Participants' peripheral blood mononuclear cells were stimulated with SARS-CoV-2 spike and nucleocapsid proteins peptides, nil control, and phytohemagglutinin control. Response was measured by an interferon-γ enzyme-linked immunosorbent spot (T-SPOT; Oxford Immunotec). (A) SARS-CoV-2–specific T-cell response of healthy controls (HCs) at 3 time points: 2 to 4 weeks after the second vaccine dose: $n = 11$, 25.4 ± 10.8; 6 months after the second vaccine dose: $n = 29$, 11 ± 5.4; and 2 to 8 weeks after the third vaccine dose: $n = 23$, 26.6 ± 15. (B) SARS-CoV-2–specific T-cell response of patients with multiple sclerosis (pwMS) treated with ocrelizumab (OCR) at 3 time points: 2 to 4 weeks after the second vaccine dose: $n = 22$, 27.4 ± 22.1; 6 months after the second vaccine dose: $n = 19$, 16.3 ± 9.2; and 2 to 8 weeks after the third vaccine dose: $n = 23$, 30.3 ± 21. (C–E) Cytokine secretion following stimulation with SARS-CoV-2 spike and nucleocapsid peptides. Cytokine analysis was performed after reduction of response to nucleocapsid peptides for each participant. In OCR-treated pwMS, interleukin (IL)-2, IL-6, and tumor necrosis factor α (TNFα) levels followed the same pattern as the interferon-γ (IFNγ)-secreting cells, decreased at 6 months after the second vaccine dose and increased with the third vaccine dose (IL-2: 37.9 ± 63.3pg/ml, 28 ± 51.7pg/ml, 35.6 ± 31.7pg/ml, $p = 0.2$; IL-6: 6,426 ± 7,878pg/ml, 4,913 ± 4,643pg/ml, 6,523 ± 6,172pg/ml, $p = 0.34$; TNFα: 44.5 ± 83pg/ml, 29.9 ± 59.8pg/ml, 99.2 ± 183.8pg/ml, $p = 0.6$). In HCs, IL-2 and IL-6 levels followed the same pattern of decreased levels 6 months after the second vaccine dose and increased levels following the third vaccine (IL-2: 26 ± 36.2pg/ml, 15.5 ± 14.6pg/ml, 42.9 ± 48pg/ml, $p = 0.3$; IL-6: 5,247 ± 5,028pg/ml, 4,338 ± 3,482pg/ml, 6,482 ± 6,124pg/ml, $p = 0.2$; TNFα: 2 ± 18.5pg/ml, 19.3 ± 46.7pg/ml, 63.4 ± 192pg/ml, $p = 0.2$). (F–H) Correlation between cytokine level and the level of IFNγ-secreting cells. *$p < 0.05$, ***$p < 0.001$. Data are presented as mean ± standard deviation. SFC, spot-forming cell. [Color figure can be viewed at www.annalsofneurology.org]

Results

Participants

Participants consisted of group of 40 HCs (mean ± SD age = 43 ± 14.8 years, 24 females) and 33 pwMS treated with OCR (mean ± SD age = 47.8 ± 13.5 years, disease duration = 13.2 ± 11.4 years, 23 females, 19 relapsing–remitting multiple sclerosis, 14 progressive multiple sclerosis; ocrelizumab treatment duration = 28.8 ± 9.6 months; time from last infusion to first and third vaccine = 4.9 ± 3.4 and 5 ± 2 months) who received 3 doses of the BNT162b2 vaccine. The first 2 doses were given 3 weeks apart, and the third was administered 5 to 6 months after the second dose.

SARS-CoV-2 mRNA Vaccine Antibody Response following 3 Doses

Following our publication of attenuated antibody response to SARS-CoV-2 mRNA vaccine in pwMS treated with OCR,[1] we evaluated longitudinally the serology response at 6 months after the second dose and 2 to 8 weeks after the third dose. We observed a significant decline in antibody levels 6 months

after the second dose, in both HCs (14,352 ± 9,453AU/ml, 1,241 ± 885AU/ml, $p < 0.0001$) and in pwMS treated with OCR (307.6 ± 604.2AU/ml, 121.2 ± 329.2AU/ml, $p < 0.0001$). The third dose increased antibody titers, above 2 to 8 weeks after the second vaccine in HCs (25,851 ± 11,302AU/ml, $p < 0.0001$) and overall in pwMS treated with OCR (1,413.2 ± 3,742AU/ml, $p = 0.9$, $p = 0.007$; Fig 1B) (Fig 1A, B) but in particular in 6 of 9 initially seropositive patients (814 ± 609.4AU/ml vs 6,252 ± 6,056AU/ml, $p = 0.05$, 2–8 weeks after the second and after the third dose, respectively). Levels remained positive in 3 of 9 initially seropositive patients. Three patients with borderline titers and 15 patients with negative antibody response to the first 2 doses remained negative following the third dose (Fig 1C, D). Titers and response rate after the third dose were significantly lower in pwMS treated with OCR compared to HCs ($p < 0.001$; 9/27 [33.3%] vs 30/30 [100%] for OCR vs HCs, respectively). All patients with increased antibody levels after the third dose were initially vaccinated ≥5 months after the last OCR infusion. Moreover, patients who were vaccinated with a third dose ≥5 months following the last OCR infusion had a significantly increased likelihood of a positive serology response (8/9 [88.9%] vs 6/17 [35.5%], $\chi^2 = 4$, $p = 0.04$; see Fig 1E, F).

Decreased SARS-CoV-2–Specific T Cells 6 Months following Vaccination Are Boosted with a Third Dose

T-cell response decreased significantly 6 months following the second vaccine dose (HC: 25.4 ± 10.8SFCs, 11 ± 5.4SFCs, $p = 0.004$; OCR: 27.4 ± 22.1SFCs, 16.3 ± 9.2SFCs, $p = 0.008$) and was restored to the levels of 2 to 8 weeks after the second dose following the third dose (HC: 26.6 ± 15SFCs; OCR: 30.3 ± 21SFCs) in both groups. There were no significant differences in response rate and level between HCs and pwMS treated with OCR after the second and third doses ($p = 0.5$; Fig 2A, B).

The spike peptides induced secretion of the TH1 cytokine IL-2 and proinflammatory cytokines IL-6 and TNFα, with undetected levels of IL-4 and IL-17 and low levels of IL-10. There were no significant differences in cytokine secretion between the two groups (see Fig 2C–E). The level of IFNγ-secreting cells significantly correlated with IL-2 in both groups (OCR: $r = 0.3$, $p = 0.035$; HCs: $r = 0.5$, $p < 0.001$) and with TNFα and IL-6 levels in pwMS treated with OCR ($r = 0.35$, $p = 0.002$; $r = 0.39$, 0.01, respectively; see Fig 2F–H).

Discussion

The need for a third vaccine dose emerged when an increase in breakthrough cases was observed even after vaccination with 2 doses of mRNA vaccines. Studies revealed waning vaccine immunity over time[9] and a strong correlation between time of vaccine administration and infection incidence.[10,11]

Here, we found that SARS-CoV-2–specific T-cell responses decreased 6 months following vaccination and increased significantly after the third dose, in both HCs and pwMS treated with OCR. Antibody levels also significantly decreased 6 months after the first 2 doses in both groups, and significantly increased following the third dose in HCs and in a subset of seropositive pwMS treated with OCR. Data on the effect of a third vaccine dose on people with negative serology are scarce.[12] In organ transplantation, it has been reported that 44% of the seronegative patients before the third dose were seropositive afterward.[13] In a small cohort of rheumatology patients treated with another B-cell–depletion therapy, rituximab, vaccinated seronegative patients remained seronegative after the third vaccine dose, with preserved T-cell response.[14] We found that none of the borderline/seronegative patients developed positive serology response after the third dose. All patients with increased antibody levels after the third dose were vaccinated >5 months after the last OCR infusion, suggesting that it may be possible to optimize the antibody response to vaccination by timing vaccination with regard to the last infusion dose. None of the participants in this study had COVID-19 before either the second or third dose of the vaccine, excluding this as a factor accounting for the differences in antibody titers.

The 6-month decline and the post-booster increase in T-cell response was similar in HCs and pwMS treated with OCR, providing initial evidence that OCR may not affect the longevity of the T-cell response. Although the cutoff level for antibodies and cellular immunity that would provide protection is still unknown, the 6-month decrease in serology response appears greater than the decline in T-cell response. Although T-cells have been suggested to have a protective role, in particular in controlling disease severity,[15,16] they also seem to be less influenced by variants that escape antibody neutralization.[8] Omicron, the most recent VOC with a high number of mutations in the receptor binding domain, has been shown to have significant evasion of vaccine-induced neutralizing antibody responses.[17,18] Most of the experimental T-cell epitopes known to be targeted in vaccinated and/or previously infected subjects are unaffected by Omicron mutations.[19] This highlights the importance of T-cell response following vaccination. Evidence from breakthrough infection will help in understanding the contribution of antibodies and T cells in mediating protection from severe COVID-19. A recent study suggested that anti-CD20s–treated patients may be at higher risk of COVID-19 infection.[20] Nevertheless, the cases were mostly mild, with most patients not requiring hospitalization; none required intensive care unit admission, and none died.

Our findings show that approximately 6 months after 2 doses of BNT162b2, both HCs and pwMS treated

with OCR had decreased levels of SARS-CoV-2 antibodies and T-cells and underscore the benefit of a third dose boosting the immune response. Follow-up studies will help address whether a third dose provides enhanced long-term protection effect on the quality of the immune response and waning response rate compared to 2 doses, in the general population and in pwMS.

Limitations of the study include small sample size and short study duration following the third vaccine. Larger studies analyzing breakthrough infection and severity of clinical outcome in patients with positive and negative serology response, including assessment of T-cell response, will provide valuable information to understand the relative protection provided by the different immune components. OCR-treated patients with additional indicators that may put them at risk of developing severe COVID-19 (eg, higher Expanded Disability Status Scale, older age, comorbidities) may benefit from the use of prophylactic monoclonal antibodies.

In conclusion, a third vaccine dose restored T-cell levels in both pwMS treated with OCR and HCs, supporting the administration of a third vaccine dose in OCR-treated patients. For seronegative patients, a third dose did not increase antibody titers. A small fraction of OCR-treated patients who were vaccinated >5 months after the last OCR infusion had boosted antibody titers. Additional studies including timing of vaccination and infusions are needed to optimize vaccine response in patients treated with anti-CD20s.

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Author Contributions

L.B., A.V.-D., and C.R. contributed to conception and design of the study; L.B., A.R., O.Z., and E.O.-D. contributed to acquisition and analysis of data; L.B., A.V.-D., C.R., N.L., and D.G.W. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

C.R. is an employee and shareholder of F. Hoffmann-La Roche. A.V.-D. reported grants from F. Hoffmann-La Roche during the conduct of the study.

References

1. Brill L, Rechtman A, Zveik O, et al. Humoral and T-cell response to SARS-CoV-2 vaccination in patients with multiple sclerosis treated with ocrelizumab. JAMA Neurol 2021;78:1510–1514.
2. Apostolidis SA, Kakara M, Painter MM, et al. Cellular and humoral immune responses following SARS-CoV-2 mRNA vaccination in patients with multiple sclerosis on anti-CD20 therapy. Nat Med 2021;27:1990–2001.
3. Sabatino JJ, Mittl K, Rowles W, et al. Impact of multiple sclerosis disease-modifying therapies on SARS-CoV-2 vaccine-induced antibody and T cell immunity. medRxiv 2021; 2021.09.10.21262933.
4. Achiron AMM, Dreyer-Alster S, et al. Humoral immune response to COVID-19 mRNA vaccine in patients with multiple sclerosis treated with high-efficacy disease-modifying therapies. Ther Adv Neurol Disord 2021;14:17562864211012835.
5. Ali A, Dwyer D, Wu Q, et al. Characterization of humoral response to COVID mRNA vaccines in multiple sclerosis patients on disease modifying therapies. Vaccine 2021;39:6111–6116.
6. Wang P, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021;593:130–135.
7. Jordan SC, Shin BH, Gadsden TM, et al. T cell immune responses to SARS-CoV-2 and variants of concern (Alpha and Delta) in infected and vaccinated individuals. Cell Mol Immunol 2021;18:2554–2556.
8. 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.
9. Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. N Engl J Med 2021;385:e84.
10. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. N Engl J Med 2021;385:1393–1400.
11. Patalon T, Gazit S, Pitzer VE, et al. Odds of testing positive for SARS-CoV-2 following receipt of 3 vs 2 doses of the BNT162b2 mRNA vaccine. JAMA Intern Med 2022;182:179–184.
12. Achtnichts L, Jakopp B, Oberle M, et al. Humoral immune response after the third SARS-CoV-2 mRNA vaccination in CD20 depleted people with multiple sclerosis. Vaccines (Basel) 2021;9:1470.
13. Kamar N, Abravanel F, Marion O, et al. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. N Engl J Med 2021;385:661–662.
14. Felten R, Gallais F, Schleiss C, et al. Cellular and humoral immunity after the third dose of SARS-CoV-2 vaccine in patients treated with rituximab. Lancet Rheumatol 2022;4:e13–e16.
15. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 2020;183:158–168.e14.
16. Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol 2022;23:186–193.
17. Van Der Straten K, Guerra D, Van Gils MJ, et al. Mapping the antigenic diversification of SARS-CoV-2. 2022.
18. Cameroni E, Saliba C, Bowen JE, et al. Broadly neutralizing antibodies overcome SARS-CoV-2 omicron antigenic shift. Nature 2022;602(7898):664–670.
19. Ahmed SF, Quadeer AA, McKay MR. SARS-CoV-2 T cell responses elicited by COVID-19 vaccines or infection are expected to remain robust against omicron. Viruses 2022;14:79.
20. Rose DR, Mahadeen AZ, Carlson AK, et al. Clinical features and outcomes of COVID-19 despite SARS-CoV-2 vaccination in people with multiple sclerosis. Mult Scler J Exp Transl Clin 2021;7:20552173 2110571.