Cellular and Humoral Immunity to SARS-CoV-2 Infection in Multiple Sclerosis Patients on Ocrelizumab and Other Disease-Modifying Therapies: A Multi-Ethnic Observational Study

Ilya Kister, MD,[1] Yury Patskovsky, PhD,[2] Ryan Curtin, BS,[2] Jinglan Pei, PhD,[3] Katherine Perdomo, BA,[1] Zoe Rimler, BS,[1] Iryna Voloshyna, PhD,[2] Marie I. Samanovic, PhD,[4] Amber R. Cornelius, MS,[4] Yogambigai Velmurugu, PhD,[2] Samantha Nyovanie, MPhil,[2] Joseph J. Kim, MS,[2] Ethan Tardio, BS,[2] Tamar E. Bacon, BA,[1] Lana Zhovtis Ryerson, MD,[1] Pranil Raut, MS,[3] Rosetta Pedotti, PhD,[5] Kathleen Hawker, MD,[3] Catarina Raposo, PhD,[5] Jessica Priest, PharmD,[3] Mark Cabatingan, PhD,[3] Ryan C. Winger, PhD,[3] Mark J. Mulligan, MD,[4] Michelle Krogsgaard, PhD,[2*] and Gregg J. Silverman, MD[6*]

Objective: The objective of this study was to determine the impact of multiple sclerosis (MS) disease-modifying therapies (DMTs) on the development of cellular and humoral immunity to severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection.
Methods: Patients with MS aged 18 to 60 years were evaluated for anti-nucleocapsid and anti-Spike receptor-binding domain (RBD) antibody with electro-chemiluminescence immunoassay; antibody responses to Spike protein, RBD, N-terminal domain with multiepitope bead-based immunoassays (MBI); live virus immunofluorescence-based microneutralization assay; T-cell responses to SARS-CoV-2 Spike using TruCulture enzyme-linked immunosorbent assay (ELISA); and IL-2 and IFNγ ELISpot assays. Assay results were compared by DMT class. Spearman correlation and multivariate analyses were performed to examine associations between immunologic responses and infection severity.
Results: Between January 6, 2021, and July 21, 2021, 389 patients with MS were recruited (mean age 40.3 years; 74% women; 62% non-White). Most common DMTs were ocrelizumab (OCR)—40%; natalizumab —17%, Sphingosine 1-phosphate receptor (S1P) modulators −12%; and 15% untreated. One hundred seventy-seven patients (46%) had laboratory evidence of SARS-CoV-2 infection; 130 had symptomatic infection, and 47 were asymptomatic. Antibody

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Address correspondence to Kister, Department of Neurology, New York University Grossman School of Medicine, New York, NY 10016. E-mail: ilya.kister@nyulangone.org and Krogsgaard, Department of Pathology, New York University Grossman School of Medicine, New York, NY 10016. E-mail: michelle.krogsgaard@nyulangone.org and Silverman, Department of Medicine, NYU Grossman School of Medicine, 435 E 30th Street, Room 517, New York, NY 10016. E-mail: gregg.silverman@nyulangone.org

*These authors should be considered co-authors.

From the [1]Department of Neurology, NYU Multiple Sclerosis Comprehensive Care Center, New York University Grossman School of Medicine, New York, NY; [2]Department of Pathology, Laura and Isaac Perlmutter Cancer Center, New York University Grossman School of Medicine, New York, NY; [3]Genentech, Inc., South San Francisco, CA; [4]Department of Medicine, NYU Langone Vaccine Center, NYU Grossman School of Medicine, New York, NY; [5]F. Hoffmann-La Roche Ltd, Basel, Switzerland; and [6]Division of Rheumatology, Department of Medicine, New York University Grossman School of Medicine, New York, NY

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responses were markedly attenuated in OCR compared with other groups ($p \leq 0.0001$). T-cell responses (IFNγ) were decreased in S1P ($p = 0.03$), increased in natalizumab ($p < 0.001$), and similar in other DMTs, including OCR. Cellular and humoral responses were moderately correlated in both OCR ($r = 0.45$, $p = 0.0002$) and non-OCR ($r = 0.64$, $p < 0.0001$). Immune responses did not differ by race/ethnicity. Coronavirus disease 2019 (COVID-19) clinical course was mostly non-severe and similar across DMTs; 7% (9/130) were hospitalized.

Interpretation: DMTs had differential effects on humoral and cellular immune responses to SARS-CoV-2 infection. Immune responses did not correlate with COVID-19 clinical severity in this relatively young and nondisabled group of patients with MS.

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Multiple sclerosis (MS) is treated with disease-modifying therapies (DMTs), some of which may impair immune responses to the pandemic severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) infection. The commonly used anti-CD20 therapies (aCD20) are associated with reduced antibody titers following SARS-CoV-2 infection and vaccination,[1–10] likely due to depletion of peripheral B cells that would otherwise be available for recruitment into germinal centers for antigen-mediated activation and clonal expansion. The T-cell compartment is relatively unaffected by aCD20 as only a small subset of CD20-bearing CD3+ lymphocytes are removed by aCD20.[11,12] Overall, T-cells counts and functional responses remain intact,[13,14] and, accordingly, Spike protein-specific T-cell responses to coronavirus disease 2019 (COVID-19) vaccination in aCD20-treated patients are robust.[11,15–19] T-cell responses following natural infection in aCD20-treated patients have received less attention, but are an active area of investigation.[20,21] There is very limited data on immune responses to SARS-CoV-2 infection under other commonly used DMTs, such as sphingosine-1-phosphate receptor modulators (S1P), which interfere with T-cell egress from lymphoid tissue, and fumarates, which induce mild–moderate lymphopenia.[20,22,23] Understanding the impact of DMTs on the immune response to SARS-CoV-2 infection is critical for counseling patients with MS about COVID-19 risks and determining whether a patient who experienced COVID-19 on a particular DMT is likely to derive a similar degree of protective immunity as untreated individuals.[24–27]

To address the knowledge gaps, we designed a prospective study with the goals of: (1) determining the impact of ocrelizumab (OCR) and other DMTs on the development of cellular and humoral immune memory to SARS-CoV-2 natural infection; (2) characterizing the relationship between humoral and cellular post-infection immune responses in patients with and without peripheral B-cell depletion; and (3) investigating the relationship between the clinical severity of COVID-19 and immune responses to SARS-CoV-2 in patients with MS with different DMTs. We recruited a large, ethnically diverse group of patients with MS from the New York University Multiple Sclerosis Care Center in New York City, New York, one of the epicenters of the COVID-19 pandemic in 2020 to 2021,[28,29] and comprehensively characterized humoral and cellular responses to SARS-CoV-2 using several complementary antibody and T-cell SARS-CoV-2—specific assays. A notable strength of our study is the inclusion of a large number of non-White patients—over 60% of all patients, which allowed us to investigate immune responses to SARS-CoV-2 in patients with MS from under-represented racial/ethnic groups.

Patients and Methods

Study Population

Patients seen for routine visits at the New York University Multiple Sclerosis Comprehensive Care Center in New York City (New York) were invited to participate if they had clinician-diagnosed MS (revised 2017 McDonald criteria)[30]; were treated with a US Food and Drug Administration (FDA)-approved DMT for MS, or were on no treatment; were aged 18 to 60 years; had Expanded Disability Status Scale (EDSS) score of 0 (normal) to 7 (wheelchair-bound). Exclusion criteria were: concurrent immunosuppressive therapy; active systemic cancer; primary or acquired immunodeficiency (unrelated to DMT); active drug or alcohol abuse; aCD20 therapy other than OCR; uncontrolled diabetes mellitus; end-organ failure (cardiac, pulmonary, renal, and hepatic); systemic lupus erythematosus, or other systemic autoimmune disease. Patients were also excluded if they received high-dose oral or parenteral corticosteroids, intravenous immunoglobulin (IVIG), plasmapheresis (PLEX), or convalescent plasma or polyclonal antibody treatments for COVID-19 within 3 months of sample collection; or if they had COVID-19 symptom onset or tested positive by SARS-CoV-2 real-time polymerase chain reaction (PCR) within 2 weeks of sample collection. At the time of sample collection all patients were unvaccinated for COVID-19.

All patients were interviewed by a trained research coordinator with a structured instrument. Patients were queried about each of COVID-19 symptoms listed in Centers for Disease Control and Prevention (CDC) clinical case definition[31] and any COVID-19 exposures from February 2020 to the time of enrollment; commercial SARS-CoV-2 test results (PCR or antibody) prior to enrollment; COVID-19 treatments and vaccinations; MS

treatment at the time of enrollment and infection (where applicable). Electronic medical records were reviewed for COVID-19 and MS-relevant information. COVID-19 history at enrollment was classified as "Laboratory-supported COVID infection at enrollment" if the patient met CDC clinical definition for COVID-19 and had positive commercial SARS-CoV-2 PCR or antibody test at any time prior to enrollment. If the patient met the CDC clinical case definition for COVID-19 but did not have commercial laboratory confirmation at the time of enrollment, their status was designated as "Suspected COVID-19 infection on enrollment." Patients without clinical symptoms to suggest prior COVID-19 were classified as "Non-suspected" for COVID-19 infection. Patients' final SARS CoV-2 infection status (previously infected vs non-infected) was determined based on laboratory evidence of infection prior to enrollment or serologic tests conducted during the study, as described in the next section.

Serological Analyses

Patients' serologic status was assessed using 3 different methods:

1. Electro-chemiluminescence immunoassay using the Elecsys platform (Roche Diagnostics GmbH, Mannheim Germany), measuring antibodies to nucleocapsid (N) (qualitative) and receptor binding domain (RBD) of Spike (S) protein (quantitative). All samples were processed and measured by a specialized laboratory according to the manufacturers' instructions. Values ≥1.0 U/ml were interpreted as "positive" for anti-N SARS-CoV-2 antibodies. For anti-Spike Abs, values of >0.4 U/ml were considered "positive," and those below the lower limit of quantification of the assay (<0.4 U/ml) were considered "negative" and set to 0.4 U/ml, as per the manufacturer's recommendations.[32] The levels of antibodies were expressed in U/ml, which are considered equivalent to binding antibody units (BAUs)/ml (Elecsys S units = 0.972 × BAU), as defined by the first World Health Organization (WHO) International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code 20/136).[32]
2. New York University proprietary custom multiepitope bead-based immunoassay (MBI), which measures antibody responses to three recombinant proteins (Wuhan variant total Spike, RBD, and the Spike amino-terminal domain (N-terminal domain [NTD]); Sino Biological catalog number 40590-V08B, 40592-V08B, and 40591-V49H-B, respectively), using control analytes of human serum albumin (HSA), tetanus toxoid and anti-human IgG (Jackson Immunoresearch, West Grove, PA, USA) coupled to commercial paramagnetic beads (MagPix, Luminex), as adapted from the manufacturer's instructions as previously described.[33,34] Positivity of individual MBI was set as the 3SD above the mean of pre-pandemic healthy adult controls. Assay reactivity was also confirmed with non-autoimmune serum from individuals with PCR-documented prior COVID-19 infection (samples provided by the New York University COVID-19 Bio Repository). MBI data for Spike, RBD, and NTD for healthy control and patients with COVID-19 specimens and respective positivity cutoffs are shown in Figure S1. Serologic confirmation of prior SARS-CoV-2 infection ("MBI seropositive") was defined conservatively as two or more independent Spike, RBD, and NTD positive assay results.
3. SARS-CoV-2 viral neutralization activity of plasma was measured in an immunofluorescence-based assay that detects the neutralization of infectious virus (SARS-CoV-2 isolate USA-WA1/2020 (NR-52281, GenBank accession no. MT233526) in cultured Vero E6 cells (African Green Monkey Kidney; ATCC #CRL-1586) as described in detail in Ref.35 All SARS-CoV-2 infection assays were performed in the BSL3 facility of New York University Grossman School of Medicine (New York, NY).

Assays of SARS-CoV-2- Specific T-Cell Response

T-cell responses to SARS-CoV-2 Spike protein were assessed in whole blood samples with TruCulture stimulation system (Rules Based Medicine, Austin, TX, USA) according to the manufacturer's instructions. Whole blood samples were incubated for 48 hours at 37°C. Collected supernatants were analyzed with IFNγ and IL-2 enzyme-linked immunosorbent assay (ELISA) assay for measuring cytokine production following the manufacturer's protocol (Thermo Fisher [Waltham, MA, USA]; Catalog # ENEHIFNG and 50-112-5363, respectively). The response for the TruCulture system was conservatively defined as positive if both IFNγ and IL-2 assays were at the level of ≥1 pg/ml.

Cellular responses to SARS-CoV-2 were also characterized by quantification of IFNγ and IL-2 producing cells by ELISpot for a subset of patients to corroborate results obtained with the TruCulture system. Briefly, peripheral blood mononuclear cells (PBMCs) were in vitro stimulated with 1 μg/peptide/ml SARS-CoV-2 peptide pool, consisting of Spike, N, and M proteins 15-mer peptide PepTivator libraries (Miltenyi Biotec, Auburn, CA, USA) for 48 hours. The number of activated T-cells were detected with ImmunoSpot IFNγ and IL-2 kits (Cellular Technology Limited [Shaker Heights, OH, USA], Cat # hIFNgp-2M/10 and hIL2p-2M/10) according to the manufacturer's instructions, and spots were counted using a CTL S6 EM2 ELISpot reader (Immunospot). Positive

results were confirmed by repeated ELISpot assays. The results were expressed as spot-forming unit (sfu) per 10^6 PBMCs. Responses were considered positive if the results were at least three times the mean of the negative control wells and >25 sfu per 10^6 PBMCs. Human CEF (cytomegalovirus [CMV], Epstein–Barr virus, and influenza virus) peptide pool (3615–1; MabTech, Stockholm, Sweden) and 1 μg/ml phytohemagglutinin-L (PHA-L; Sigma Aldrich, St. Louis, MO, USA) were used as positive controls. The negative control contained PBMC, and the corresponding cell culture medium was used to determine the background signal.

Statistical Analyses

All patients were included in the analyses. Descriptive summaries of the results from the immunoassays were reported for continuous and categorical variables. Results that have heavily skewed distributions were normalized by log transformation. For continuous variables, mean, standard deviation (SD), median, and range were reported. For categorical variables, counts and percentage of patients with positive results were summarized. Correlation analyses were performed using the Spearman correlation. Comparisons of end points were performed among patients on the various DMTs and untreated patients (no DMT). Multivariate analyses were performed to account for possible confounding characteristics, including the patients' COVID-19 clinical severity and MS treatments. Missing data were not imputed.

The study was approved by the New York University Grossman School of Medicine institutional review board (IRB).

Results

Demographic and Clinical Characteristics of the Patients

From January 6, 2021, to July 21, 2021, 389 non-vaccinated patients with MS were recruited. Demographic and clinical characteristics, DMT use, and COVID-19-relevant comorbidities of the patients are shown in Table 1. The patients were relatively young (mean age = 40.3 ± 10.8 years, range = 18–60 years), non-disabled (68% fully ambulatory), and otherwise healthy (68% without any COVID-19-relevant comorbidities). Sex ratio (74% women) and race/ethnic composition (62% non-White) of the patients are representative of our clinic population.[36] "COVID history at enrollment" was classified as "laboratory-supported COVID at enrollment" in 101 patients (26% of all patients), "suspected COVID at enrollment" (symptoms only) in 76 patients (20%), and "COVID non-suspected" in 212 patients (54%). These 3 subgroups had similar demographic and clinical MS characteristics (data not shown).

TABLE 1. Demographic and Clinical Characteristics of Patients with MS

	All patients, N = 389
Age, yr	
Mean (SD)	40.3 (10.8)
Median (Q1, Q3)	40.0 (32.0, 49.0)
Female, n (%)	286 (73.5)
Race/ethnicity, n (%)	
White	147 (37.8)
African American/Black	111 (28.5)
Hispanic	106 (27.2)
Other	25 (6.4)
DMT at enrollment, n (%)	
Ocrelizumab	154 (39.6)
Natalizumab	65 (16.7)
No DMT	58 (14.9)
S1P	48 (12.3)
Fumarates	34 (8.7)
Glatiramer acetate	11 (2.8)
Teriflunomide	10 (2.6)
Interferon β	9 (2.3)
Ambulatory status, n (%)	
Fully ambulatory	265 (68.1)
Impaired but no assistance	49 (12.6)
Assistance with cane	45 (11.6)
Assistance with walker	26 (6.7)
Non-ambulatory/wheelchair	4 (1.0)
Number of comorbidities[a] n (%)	
0	264 (67.9)
1	94 (24.2)
2	24 (6.2)
3	7 (1.8)

S1P receptor modulators included Fingolimod (Gilenya), Siponimod (Mayzent), Ozanimod (Zeposia); Fumarates include Dimethyl Fumarate (Tecfidera), diroximel fumarate (Vumerity); Interferon β included Interferon beta-1a (Avonex, Rebif) and Interferon beta-1b (Betaseron).

[a]COVID-relevant comorbidities included: hypertension, chronic obstructive pulmonary disease, cardiovascular disease, diabetes mellitus, obesity, sickle cell disease, chronic kidney disease, chronic liver disease, and (non-skin) cancer.

DMT = disease-modifying therapy; MS = multiple sclerosis; S1P = sphingosine 1-phosphate receptor modulators; SD = standard deviation Q1 (quartile 1) and Q3 (quartile 3) represent median of the lower and upper half of the data.

SARS-CoV-2 Antibody Testing

All patients underwent serologic testing by Elecsys assay for antibodies to N and the Spike RBD, and by MBI for whole Spike protein, and the Spike RBD and NTD components of Spike. There was a strong correlation between the individual assays for the whole Spike and Spike components by MBI ($r = 0.77–0.82$, $p < 0.0001$), and between anti-RBD antibody levels by MBI and by Elecsys ($r = 0.69$, $p < 0.0001$). SARS-CoV-2 antibody responses did not differ by race/ethnicity (White vs Black vs Hispanics vs other) with either Elecsys or MBI assay systems (Table S1).

The sensitivity and specificity of 2 antibody assays are summarized in Table 2. The patients with laboratory-supported COVID at enrollment are shown in the first column of Table 2. Despite the more stringent definition of seropositivity for MBI (≥2 of 3 independent antibody assays with >3 SD above pre-pandemic means) than Elecsys (either one of the 2 antibodies was positive), MBI had greater sensitivity for antibody detection in patients with laboratory-confirmed COVID-19 on enrollment: 92% for MBI versus 81% for Elecsys. Eight patients were considered to have false-negative results by MBI, as they had a laboratory confirmed infection before enrollment by commercial tests but were negative by MBI; all 8 patients were on OCR at the time of infection.

The second column of Table 2 presents the prevalence of SARS-CoV-2-specific antibodies in patients with suspected COVID on enrollment (symptoms only) and the third column—in non-suspected cases. In the suspected COVID at enrollment group, MBI identified 29 out of 76 as "MBI-seropositive" (≥2 of 3 independent antibody assays with >3 SD above pre-pandemic mean levels) and in the non-suspected group, MBI identified 47 out of 212 as MBI-seropositive. Thus, there was a total of 76 patients from "suspected" and "non-suspected" subsets who had strong serologic evidence of SARS-CoV-2 infection. Of these patients, half (n = 38) were also positive by Elecsys. In contrast, Elecsys identified only a single case that was negative by MBI. Overall, MBI's greater sensitivity for antibody detection proved useful in the study of immune responses to chronologically remote SARS-CoV-2 infections, especially in immunosuppressed individuals.

Defining the Subset of Patients with Prior SARS-CoV-2 Infection

In addition to the 101 patients with laboratory supported COVID-19 on enrollment, we identified 76 patients who did not have laboratory testing for SARS CoV-2 prior to enrollment, but tested positive on at least 2 out of 3 independent antibody levels on MBI and were thus considered to have had SARS-CoV-2 infection. Thus, the total

TABLE 2. Seropositivity Rates by Elecsys and MBI Stratified by COVID History on Enrollment

	Laboratory supported COVID on enrollment, n = 101	Suspected COVID on enrollment, n = 76	Not suspected on enrollment, n = 212
Elecsys, n (%)			
N	67[a] (67.0)	12 (15.8)	19 (9.0)
S	80 (79.2)	16 (21.1)	23 (10.8)
Elecsys Seropositivity	**82 (81.2)**	**16 (21.1)**	**23 (10.8)**
MBI, n (%)			
Spike	93 (92.1)	32 (42.1)	47 (22.2)
RBD	95 (94.1)	29 (38.2)	48 (22.6)
NTD	91 (90.1)	31 (40.8)	80[b] (38.5)
MBI Seropositivity	**93 (92.1)**	**29 (38.2)**	**47 (22.2)**

"Laboratory supported COVID on enrollment" is defined as "clinical symptoms consistent with COVID (CDC clinical case definition) and laboratory confirmation of SARS-CoV-2 prior to enrollment." "Suspected COVID on enrollment" is defined as "clinical symptoms consistent with COVID (CDC clinical case definition) but no laboratory confirmation of SARS-CoV-2 prior to enrollment." "Not suspected on enrollment" is defined as not meeting CDC clinical case definition. "Elecsys seropositivity" is defined as "'either N (nucleocapsid) or S (spike) antibody positive'" as defined by manufacturer. "MBI seropositivity" is defined more stringently as "'≥2 or 3 independent antibody assays >3 SD over the mean pre-pandemic levels.'"

[a]Denominator = 100.

[b]Denominator = 208.

CDC = Centers for Disease Control and Prevention; DMT = XXX; MBI = multiepitope bead-based immunoassay; N = nucleocapsid; NTD = N-terminal domain; RBD = receptor-binding domain; S = spike; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus 2.

number of patients with test-supported SARS-CoV-2 infection in our group was 177, or 46% of all patients.

The relationship between COVID-19 status at enrollment and the final SARS-CoV-2 infection status is shown in the Sankey diagram in Figure 1. Patients with prior SARS-CoV-2 infection included 130 symptomatic cases and 47 asymptomatic cases. The asymptomatic cases constituted 27% of all SARS-CoV-2-infected patients and had similar demographic and MS-related characteristics as the symptomatic cases (data not shown). The majority of patients with asymptomatic prior infection, in addition to testing positive on 2 or more serologies by MBI, had additional collateral evidence of past infection: 23 of 47 patients (49%) tested seropositive on one or both Elecsys antibody assays; 8 of 47 patients (17%) had positive COVID-specific T-cell responses (see Section Cellular Responses to SARS-CoV-2 Antigens in Patients with Prior SARS CoV-2 Infection and in SARS-CoV-2 Seronegative Patients); and 10 of 47 patients (21%) reported a history of close exposure to COVID-19 infected individuals at home or work, or were essential personnel with a high risk of exposure.

Timing of Infection and Clinical Outcomes in Patients with Prior SARS-CoV-2 Infection Stratified by DMT

Mean time from symptomatic infection to blood collection was 34.5 ± 19.0 weeks (range = 4.3–70.4 weeks). The calendar time distribution of symptomatic infections in shown in Figure S2. The bimodal distribution matches the epidemiology of the spread of COVID-19 in New York City.[37] All infections occurred before the spread of Delta and Omicron variants. Among the 130 patients with symptomatic COVID-19, 54 (42%) had respiratory symptoms (shortness of breath or difficulty breathing), 9 (7%) required hospitalization, and 2 were subsequently admitted to the intensive care unit (ICU). The hospitalized patients (n = 9) were, on average, 51.2 years old and 7 of 9 patients (78%) had COVID-19-relevant comorbidities, whereas non-hospitalized patients were, on average, 39.3 years old and only 31% had comorbidities. The 2 patients admitted to the ICU were a 40-year-old man with no comorbidities on dimethyl fumarate at the time of infection and a 48-year-old woman with

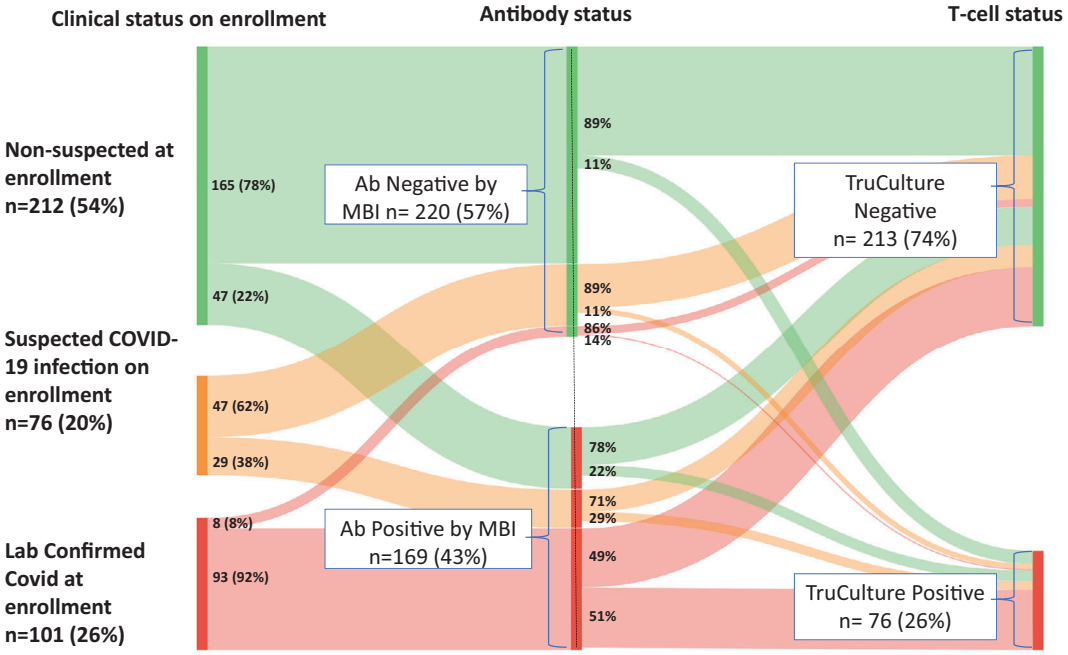

FIGURE 1: COVID-19 history at enrollment (*left panel*) stratified by MBI serostatus (*middle*) and TruCulture (*right*). Sankey diagram illustrates proportions of patients with "laboratory-supported COVID at enrollment," "suspected COVID at enrollment," and "COVID not suspected at enrollment" who tested positive by MBI (*middle panel*) and proportion of MBI-positive and MBI-negative patients who tested positive on Truculture (see Section Cellular Responses to SARS-CoV-2 Antigens in Patients with Prior SARS CoV-2 Infection and in SARS-CoV-2 Seronegative Patients). "Laboratory supported COVID on enrollment" is defined as "clinical symptoms consistent with COVID-19 (CDC clinical case definition) and laboratory confirmation of SARS-CoV-2 prior to enrollment." "Suspected COVID on enrollment" is defined as "clinical symptoms consistent with COVID-19 (CDC clinical case definition) but no laboratory confirmation of SARS-CoV-2 before enrollment." "Not suspected on enrollment" is defined as not meeting CDC clinical case definition. "MBI seropositive" is defined as ≥2 or 3 independent antibody assays >3 SD over the mean pre-pandemic levels. "TruCulture positive" was defined as both IFNγ and IL-2 assays were at the level of ≥1 pg/ml. CDC = Centers for Disease Control and Prevention; COVID-19 = coronavirus disease 2019; MBI = mean fluorescence intensity; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus 2.

TABLE 3. Clinical Characteristics of Patients with MS with Laboratory Confirmed COVID by DMT[a]

	OCR, n = 70	GA, n = 6	Interferon β, n = 4	Fumarates, n = 17	S1P, n = 19	NAT, n = 22	No DMT, n = 39	All Patients, n = 177
COVID symptoms, n (%)								
Symptomatic	49 (70.0)	5 (83.3)	2 (50.0)	17 (100.0)	12 (63.2)	17 (77.3)	28 (71.8)	130 (73.4)
Asymptomatic	21 (30.0)	1 (16.7)	2 (50.0)	0	7 (36.8)	5 (22.7)	11 (28.2)	47 (26.6)
Age, yrs, mean (SD)	37.9 (9.7)	40.0 (13.0)	49.0 (7.4)	42.2 (10.7)	43.0 (9.2)	36.8 (11.4)	41.9 (12.6)	39.9 (10.9)
Female, n (%)	53 (75.7)	4 (66.7)	2 (50.0)	10 (58.8)	16 (84.2)	15 (68.2)	25 (64.1)	125 (70.6)
Race, n (%)								
White	27 (38.6)	3 (50.0)	1 (25.0)	6 (35.3)	6 (31.6)	10 (45.5)	16 (41.0)	69 (39.0)
African American/Black	14 (20.0)	0	3 (75.0)	6 (35.3)	7 (36.8)	6 (27.3)	13 (33.3)	49 (27.7)
Hispanic	23 (32.9)	2 (33 3)	0	5 (29.4)	5 (26.3)	5 (22.7)	9 (23.1)	49 (27.7)
Other	6 (8.6)	1 (16.7)	0	0	1 (5.3)	1 (4.5)	1 (2.6)	10 (5.6)
Number of comorbidities[b], n (%)								
0	53 (75.7)	5 (83.3)	2 (50.0)	8 (47.1)	13 (68.4)	15 (68.2)	22 (56.4)	118 (66.7)
1	15 (21.4)	1 (16.7)	2 (50.0)	6 (35.3)	5 (26.3)	4 (18.2)	13 (33.3)	46 (26.0)
2	2 (2.9)	0	0	2 (11.8)	0	3 (13.6)	3 (7.7)	10 (5.6)
3	0	0	0	1 (5.9)	1 (5.3)	0	1 (2.6)	3 (1.7)
Symptom count, n (%)								
0 (asymptomatic)	19 (27.1)	1 (16.7)	2 (50.0)	0	7 (36.8)	5 (22.7)	9 (23.1)	43 (24.3)
1–3	17 (24.3)	3 (50.0)	0	3 (17.6)	4 (21.1)	5 (22.7)	9 (23.1)	41 (23.2)
>3	34 (48.6)	2 (33.3)	2 (50.0)	14 (82.4)	8 (42.1)	12 (54.5)	21 (53.8)	93 (52.5)
Symptom duration, wk								
Median (Q1, Q3)	1.9 (1.0, 3.0)	0.7 (0.6, 1.0)	1.6 (1.0, 2.3)	2.0 (1.1, 2.9)	1.4 (1.0, 2.0)	1.4 (1.0, 2.0)	2.6 (1.0, 6.0)	2.0 (1.0, 3.0)
Respiratory symptoms, n (%)	16 (22.9)	1 (16.7)	0	12 (70.6)	4 (21.1)	6 (27.3)	15 (38.5)	54 (30.5)
Hospitalization, n (%)	2 (2.9)	1 (16.7)	0	2 (11.8)	1 (5.3)	1 (4.5)	2 (5.1)	9 (5.1)

Laboratory confirmed COVID is defined as Lab-supported COVID on enrollment and any MBI seropositive for SARS-CoV-2 (independent of COVID history at enrollment). S1P receptor modulators included Fingolimod (Gilenya), Siponimod (Mayzent), Ozanimod (Zeposia); Fumarates include Dimethyl Fumarate (Tecfidera), diroximel fumarate (Vumerity); Interferon β included Interferon beta-1a (Avonex, Rebif) and Interferon beta-1b (Betaseron).

[a]For patients with symptomatic COVID, we use DMT at the time of symptoms; for asymptomatic patients, we use DMT at the time of enrollment. Comorbidities included hypertension, chronic obstructive pulmonary disease, cardiovascular disease, diabetes mellitus, obesity, sickle cell disease, chronic kidney disease, chronic liver disease, and cancer (non-skin cancers only).

[b]COVID-relevant comorbidities: hypertension, chronic obstructive pulmonary disease, cardiovascular disease, diabetes mellitus, obesity, sickle cell disease, chronic kidney disease, chronic liver disease, and (non-skin) cancer.

COVID = coronavirus disease; DMT = disease-modifying therapy; GA = glatiramer acetate; MBI = mean fluorescence intensity; MS = multiple sclerosis; NAT = natalizumab; OCR = ocrelizumab; S1P = sphingosine 1-phosphate receptor modulators; SARS-CoV-2 = severe acute respiratory syndrome-coronavirus 2.

3 comorbidities (obesity, cardiovascular disease, and prior cancer) on S1P at the time of infection.

Demographic, clinical, and COVID-19 characteristics—number of symptoms, presence of respiratory symptoms, symptom duration, hospitalization rates—stratified by DMT class are shown in Table 3. The percentage of asymptomatic patients and COVID-19 clinical characteristics were comparable across most DMTs. However, time from infection to sample collection was much shorter for OCR (26.7 ± 18.4 weeks) than all other patients (39.2 ± 17.9 weeks).

Humoral Responses among Patients with Prior SARS-CoV-2 Infections Stratified by DMT

Among patients with prior SARS-CoV-2 infection, the seropositivity rate by MBI was 100% for all DMTs except for OCR, for which the seropositivity rate was 89%. Seropositivity rates by Elecsys for non-OCR DMTs and the no-treatment group ranged from 83% to 100%, whereas for OCR it was only 36%.

Levels of anti-Spike antibodies by Elecsys (Fig 2A) and MBI (Fig 2B) were approximately 10-fold lower in patients treated with OCR with prior SARS-CoV-2 compared with untreated patients with prior infection. The patients with S1P had significantly lower antibody levels than the untreated patients, as measured by Elecsys, but the difference was not statistically significant with the MBI assay. For OCR-treated patients with a history of COVID-19, there was a nonsignificant trend for increased anti-SARS-CoV-2 Ab titers on MBI assay with longer time from the last OCR infusion prior to infection and infection onset ($r = 0.285$, $p = 0.064$).

Samples were available to measure functional neutralizing antibody (Nabs) titers in 77 patients with prior SARS-CoV-2 infection. Nab levels showed a strong correlation with anti-RBD antibody levels detected by MBI assay ($r = 0.71$, $p < 0.001$), yet 21% of patients with high levels of binding antibodies did not have detectable functional Nabs. Compared with untreated patients, Nab titers were marginally lower in OCR-treated patients ($p = 0.055$), and higher in Natalizumab-treated patients ($p = 0.01$; see Fig 2C).

For each patient with symptomatic COVID-19 after OCR infusion, we plotted in Figure 3 the timeline of the last OCR infusion before infection (start of the gray bar); COVID-19 symptom onset (end of the grey bar); OCR infusion following infection if any (blue circle); and time of sampling (green rhomboid); alongside their respective Nab and anti-Spike MBI levels and TruCulture IFNγ responses. Of note, 23 of 27 (85%) patients who had an infection within 6 months after OCR infusion had low or undetectable ID_{50} ≤100 Nab titers.

Multivariate analyses of all patients infected with SARS-CoV-2 showed that treatment with OCR and the absence of hypertension predicted lower Elecsys antibody (the biological significance of the latter correlation is unknown). In patients with symptomatic COVID-19, treatment with OCR and longer infection-to-collection time correlated with lower MBI levels. In the subset of previously SARS-CoV-2 infected patients who were tested for Nabs, only OCR treatment at the time of infection was a

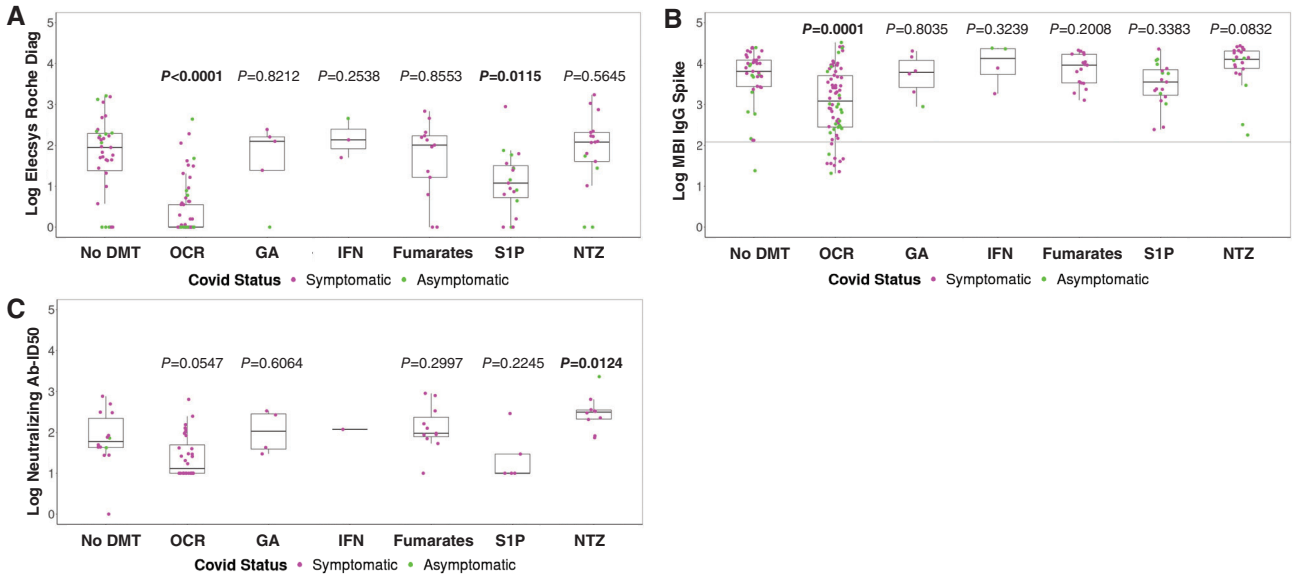

FIGURE 2: Elecsys (A), MBI (B), and Nab levels (C) anti-Spike antibodies by DMT. Symptomatic cases are shown in magenta and asymptomatic cases in green. Neutralizing antibody titers are shown as log10 of half-maximal inhibitory dilution (ID50). The *p* values <0.05 are shown in bold. Ab = antibody; COVID-19 = coronavirus disease 2019; DMT = disease-modifying therapy; GA = glatiramer acetate; ID_{50} = half-maximal inhibitory dilution; IgG = immunoglobulin; MBI = multiepitope bead-based immunoassay; Nabs = neutralizing antibodies; OCR = ocrelizumab; S1P = sphingosine 1-phosphate receptor modulators.

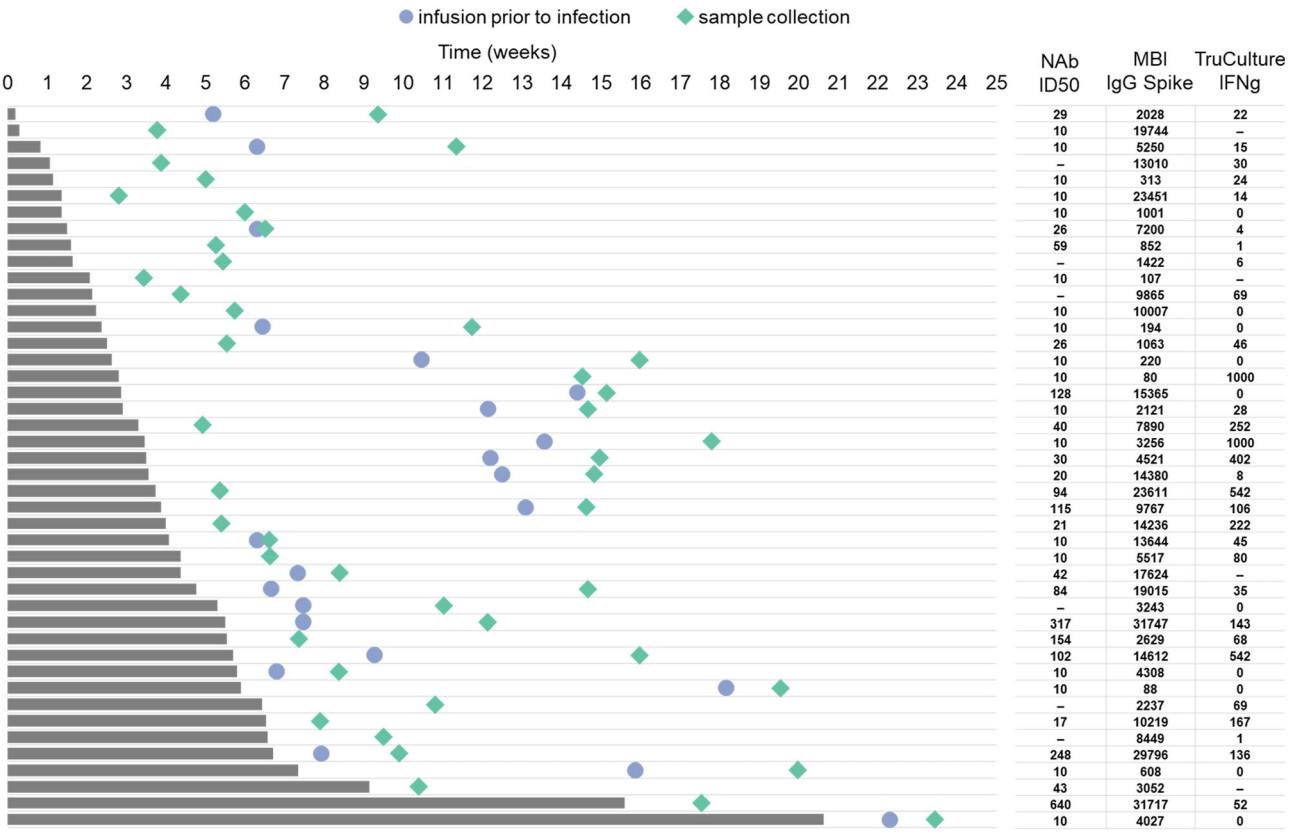

FIGURE 3: Timeline for symptomatic COVID-19 patients who were infected following OCR infusion. Timeline from last OCR infusion before infection (time zero, *start of the grey bar*) to COVID-19 infection onset (*end of the grey bar*), subsequent OCR infusion before sample collection (*blue circle*) and sample collection (*green rhombus*). Each line represents a patient's timeline. Neutralizing Ab titers, binding IgG anti-spike level by MBI and TruCulture IFNγ are shown for each patient in the respective line. Neutralizing antibody titers are shown as log10 of half-maximal inhibitory dilution (ID_{50}). COVID-19 = coronavirus disease 2019; MBI = multiepitope bead-based immunoassay; Nabs = neutralizing antibodies; OCR = ocrelizumab;

predictor of lower Nabs titers, although the number of available samples for non-OCR DMTs was limited (eg, only 5 samples for S1P).

Cellular Responses to SARS-CoV-2 Antigens in Patients with Prior SARS CoV-2 Infection and in SARS-CoV-2 Seronegative Patients

We performed in vitro T-cell stimulation studies on 159 of the 177 patients with prior SARS-CoV-2 infection (as defined in the Discussion section) using the TruCulture assay. Samples were not available or failed quality assurance check for 18 previously infected patients. Positive T-cell responses (above-zero values for both IFNγ and IL-2) were observed in 62 of 159 (39%) of all the patients with prior infection. In the subset of patients for whom IFNγ responses were tested by both TruCulture and ELISpot tested (including one patient with missing TruCulture value), the concordant positive rate was 59.6% (ie, 53/89 samples tested positive for IFNγ on both TruCulture and ELISpot); concordant negative rate was 15.7% (14/89 samples tested negative on both assays); and discordant rate 24.7% (22/89 were positive for IFNγ on TruCulture and negative on ELISpot, or vice versa). In the subset with both TruCulture and ELISpot IL-2 responses tested, concordant positive rate 36.9% (31/84 were positive for IL-2 on both assays); concordant negative rate 33.3% (28/84); and discordant rate between TruCulture and ELISPot was 29.8% (25/84 samples). Cellular responses did not differ by race/ethnicity by either TruCultre or ELISpot (see Table S1).

We also performed in vitro T-cell stimulation studies on 130 MBI-seronegative patients who did not meet our criteria for infection. TruCulture reactivity was observed in 14 of 130 (11%) of the MBI-seronegative patients. None of these 14 patients were seropositive by Elecsys assay, nor by MBI Spike or RBD, but 9 of 14 patients (65%) were seropositive by NTD (non-receptor binding domain of Spike) by MBI and 2/4 (50%) had positive ELISpot results.

SARS-CoV-2 Specific Cellular Responses in Patients with Prior COVID-19 Infection Stratified by DMT

The OCR group had similar TruCulture IFNγ responses compared to the untreated reference group, whereas S1P

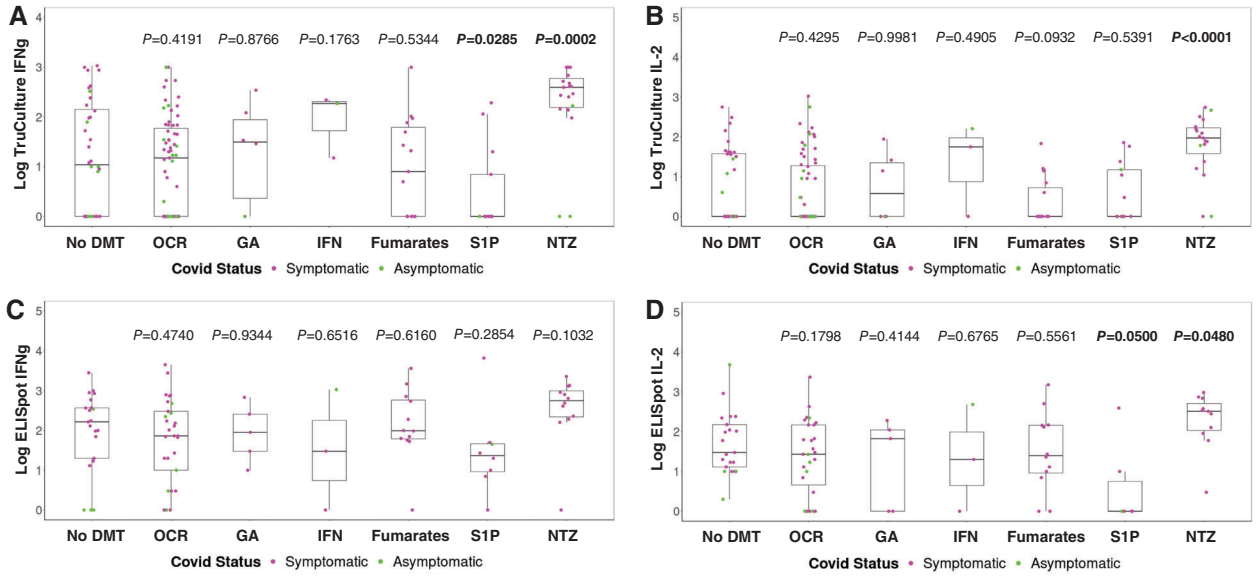

FIGURE 4: Induced T-cell activation in patients with MS grouped by DMT based on IFNγ and IL2 secretion in TruCulture system (A, B) or evaluated by ELISpot (B, C). Symptomatic cases are shown in magenta and asymptomatic cases in green. The *p* values <0.05 are shown in bold. COVID-19 = coronavirus disease 2019; DMT = disease-modifying therapy; GA = glatiramer acetate; MS = multiple sclerosis; NAT = natalizumab; OCR = ocrelizumab; S1P = sphingosine 1-phosphate receptor modulators.

showed depressed responses ($p = 0.0285$) and Natalizumab had elevated responses ($p = 0.0002$; Fig 4A). IL-2 responses by TruCulture were also elevated with Natalizumab ($p < 0.0001$), but were similar for all other DMTs, including OCR (see Fig 4B). T-cell responses assessed by ELISpot are presented in Figure 4C for IFNγ and Figure 4D for IL-2. Only Natalizumab-treated patients had marginally elevated IL-2 responses ($p = 0.048$), whereas S1P had marginally depressed IL-2 responses by ELISpot.

In patients with prior SARS-CoV-2 infection, there was no trend for decreasing cellular responses (TruCulture IFNγ) with increasing time from infection neither in the entire cohort nor in OCR subset (data not shown). The multivariate analyses did not identify any predictors of TruCulture responses.

In SARS-CoV-2 infected patients, the anti-Spike antibody by MBI and cellular IFNγ responses by TruCulture showed a moderate degree of correlation overall ($r = 0.53$, $p < 0.0001$), and in both OCR ($r = 0.45$, $p = 0.0002$; Fig S3A) and non-OCR ($r = 0.64$, $p < 0.0001$; Fig S3B) subsets.

Relationship Between COVID-19 Infection Symptoms and Immune Responses to SARS-CoV-2 in Patients on OCR and Other DMTs

In a multivariate model to predict MBI Spike levels based on DMT status and COVID-19 clinical variables (symptom duration, symptom number, and presence/absence of respiratory symptoms), only OCR treatment was a predictor for lower MBI Spike values. In a multivariate model to predict T-cell responses with TruCulture assay, longer COVID-19 symptom duration was associated with lower T-cell responses, but this relationship was driven by few outliers with "long COVID" and was not present if patients with symptoms that persisted for >1 month were excluded. In the 9 hospitalized patients, the mean anti-SARS-CoV-2 antibody values and T-cell responses were similar to the non-hospitalized group, except for TruCulture IFNγ responses that were higher in the hospitalized patients (data not shown).

Discussion

In an ethnically diverse group of 389 patients with MS from the New York University Multiple Sclerosis Care Center in New York City, 46% had laboratory evidence of prior SARS-CoV-2 infection. This prevalence is higher than what would be expected for our area based on the NYC Department of Health seroprevalence study from July 2021 (the end of our study period),[29] possibly due to over-representation in our Center of patients from Brooklyn, Queens, and Bronx neighborhoods with a very high incidence of prior SARS-CoV-2 infections (40–50%); use of highly sensitive multiplex bead-based immunoassays to measure seroprevalence; and the presumed greater motivation to participate in the study on the part of patients with suspected or known prior COVID-19. We confirmed COVID-19 diagnosis in 38% of patients with a history of COVID-19-like illness, but no commercial laboratory confirmation prior to enrollment, which is almost identical to the rate of SARS CoV-2 seropositivity among

undocumented cases in a population-based study from New York City.[38] The rate of asymptomatic infection in our patients—27%—is lower than 33% rate in 2 large European studies, but higher than the 16% among World Trade Center responders in the New York City area.[39] Our results suggest that asymptomatic SARS CoV-2 infections are not uncommon among patients with MS and occur at a rate comparable to the general population.

The high prevalence of SARS-CoV-2 infection in our patients with MS allowed us to investigate how the immune memory to SARS-CoV-2 varies depending on DMT status at the time of infection. Patients who developed the infection while on OCR had an approximately 10-fold decrease in anti-SARS-CoV-2 binding antibody levels compared to the untreated group, even though the time from infection to sample collection was, on average, 13 weeks shorter for OCR patients than all others. In multivariate analyses, OCR treatment was the single most important predictor of lower binding Ab levels. Functionally important Nabs were also depressed with OCR compared to the untreated group, and 85% of patients who had an infection within 6 months of OCR infusion generated no or very low levels of Nabs.

On the other hand, anti-viral cellular responses, assessed via the TruCulture assay and by ELISpot (for a subset of patients) were present at a similar rate in OCR and untreated patients. T cell responses were largely independent of time from the last OCR infusion prior to infection, unlike humoral responses that tended to be weaker with shorter infusion-to-infection time. The uncoupling of antibody and T-cell responses in peripherally B-cell depleted patients, recently reported by others as well,[20,21] may be due to the relative sparing of B-cells in secondary lymphoid organs, where T cell activation occurs, or to the fact that antigen-presenting function of memory B-cell is not essential for generating appropriate T-cell responses following COVID-19 infection or vaccination. Robust T-cell response has been associated with less severe disease[40–42] and may in part explain "clinical-serologic dissociation" in our mostly young and non-disabled patients: the similarity in COVID-19 severity across DMTs, despite markedly lower antibody responses with OCR compared with other DMTs.

Taken together, our data suggests that previously infected or vaccinated patients on aCD20 are less likely to be protected against "breakthrough" infection than others, a prediction borne out by a recent population-based study from the United Kingdom,[43] but are probably still protected against COVID progression due to intact T-cell immunity.[15,18,44,45] Memory T cells that contribute to protection against severe disease by eliminating infected cells and limiting viral replication may explain, at least in part, why vaccines prevent hospitalizations and deaths even against variants that exhibit limited neutralization by vaccine-induced humoral immunity.[46,47] Indeed, T-cell responses to Beta and Omicron variants have been documented following the third dose of mRNA vaccines even in aCD20-treated patients.[48]

Patients who experienced SARS-CoV-2 infection while on S1P receptor modulators tended to have depressed humoral and, to a larger extent, cellular immune responses than untreated patients. This may be a consequence of peripheral lymphopenia, disruption of immune cell traffic to and from the germinal center, or the impact of the drug on the downstream signaling pathways involved in cytokine production. T-cell responses in S1P-treated patients are also reduced following COVID-19 vaccination.[1,44,45] In another example of clinical-immunologic dissociation, COVID-19 outcomes were not worse in patients on S1P in our group or other large series.[9,49] A possible explanation is that S1P modulators may help protect against immune system hyperactivation—"cytokine storm"—and stabilize pulmonary epithelium,[50] thereby preventing more severe disease.

Natalizumab-treated patients antibody and cellular responses were on par, or better, than in untreated patients, possibly as a consequence of elevated T- and B-cell lymphocyte counts in the peripheral circulation with this therapy.[51] In patients treated with fumarates, both humoral or cellular responses were intact despite the lower peripheral lymphocyte counts with these drugs.[52] In contrast to a prior study,[20] we did not observe impairment in immune responses in IFNβ-treated patients, in line with the observation from large cohorts that COVID-19 outcomes are actually better in IFNβ-treated patients than with other DMTs.[9] In patients on glatiramer and teriflunomide, immune responses were intact, though firm conclusions cannot be made given the relatively small number of infections in each of these DMT classes (<11 patients per DMT group).

Several strengths of our study deserve mention. First, the large number of studied patients and high SARS-CoV-2 seropositive rate allowed for statistically meaningful comparisons of immunologic outcomes for the different DMTs.[20] Second, our population largely reflects the diversity of New York City, with 29% of patients self-identifying as African Americans and 27% as Hispanics. (Asians were relatively under-represented in our Center, likely due to the lower prevalence of MS in this ethnic group.) In univariate and multivariate analyses, race/ethnicity was not a predictor of clinical outcomes, nor of antibody or cellular anti-SARS-CoV-2 responses in either OCR or non-OCR patients (see Table S1). Third, due to the epidemiology of COVID-19 spread in our area—with a highly destructive first wave in Spring of 2020 followed

by a large second wave in Fall–Winter 2020—we were able to collect samples with a wide range of time-to-infection and investigate the durability of immune responses over the median interval of 43 weeks from infection (interquartile range = 14–50 weeks). Fourth, we used a custom MBI specially designed to interrogate immune responses to SARS-CoV-2 antigens with enhanced sensitivity. MBI was instrumental in identifying serologic evidence of infection in OCR-treated patients with depressed antibody responses and asymptomatic infections, which were often missed with the commercially available Elecsys test designed for high throughput in the clinical setting. Differences in seropositivity rates in OCR-treated patients with past infection (36% by Elecsys and 89% by MBI) emphasize the importance of considering assay sensitivity and the clinical context when interpreting published seroprevalence studies. The higher sensitivity of multiplex bead array over electro-chemiluminescence immunoassay has been noted by others as well.[20] Fifth, we assessed functional neutralizing responses with live virus microneutralization assays in a subset of patients with binding antibodies to SARS-CoV-2. Despite a high degree of correlation between binding (MBI IgG Spike) and neutralizing antibody levels ($r = 0.71$), 1 in 5 patients with binding antibodies had low or undetectable neutralizing antibodies. This finding calls into question over-reliance on commercially available antibody binding assays as surrogates of serologic immunoprotection. Sixth, we used a technically simple, rapid test of cellular responses to SARS-CoV-2, TruCulture assay, and showed its utility even in patients with suppressed humoral immunity. Because TruCulture is a relatively new assay that has only been used in a few published studies,[53] we corroborated TruCulture results with a conventional but more technically demanding ELISpot in a subset of patients. The 2 tests were largely concordant, though slightly better for IFNγ responses; less than 25% of patients had discordant results by the 2 assays. Specificity of TruCulture merits further investigation. We identified a group of 14 out of 130 (11%) patients who were positive on TruCulture, but serologically negative by MBI. These TruCulture-positive, MBI-seronegative patients may represent false-positive results (possibly due to cross-reaction with other coronaviruses), or true-positive results, with absent antibody titers during convalescence,[42] or "aborted infection" in which antibody responses fail to develop in the first place.[54]

Limitations of our study include the lack of SARS-CoV-2 PCR confirmation for all of our patients, especially those infected during the first wave, in which 80% of patients were not PCR-confirmed.[55] Our inclusion criteria, although intentionally broad, precluded older, systemically immunocompromised, severely disabled patients from participation. Patients who were lost due to fatal COVID-19 infection could not be accounted for in our study. Our patients largely reflect the mild range within the spectrum of SARS-CoV-2, as evidenced by the fact that only 7% of our symptomatic cases were hospitalized as compared to the average hospitalization rate of 16% across MS studies.[56] Our findings may not be generalizable to older and more disabled patients, who account for the bulk of excess morbidity and mortality in MS.[57]

The main conclusion of our study is that relatively young and otherwise healthy patients with MS generally had favorable clinical course across DMTs despite markedly impaired adaptive immune responses associated with some of the DMTs (aCD20 and S1P). To better understand the uncoupling of T-cell from antibody responses in aCD20 treated patients and to identify predictors of immune response in patients on the different DMTs, we are conducting in-depth immunophenotyping and activation-induced marker studies.

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Potential Conflicts of Interest

I.K. served on the scientific advisory board for Biogen Idec, Genentech, Alexion, EMDSerono; received consulting fees from Roche; and received research support from Guthy-Jackson Charitable Foundation, National Multiple Sclerosis Society, Biogen Idec, Serono, Genzyme, and Genentech/Roche; he receives royalties from Wolters Kluwer for "Top 100 Diagnosis in Neurology" (co-written with Jose Biller). G.J.S. received honoraria from BMS, Eli Lilly and Genentech, and research support from BMS, Genentech, Lupus Research Alliance, NIH-NIAMS, NIH-NIAID, and NIH-NILB. M.K. is on the scientific advisory board for NexImmune and Genentech and received research support from Merck Sharp &

Dohme Corp., a subsidiary of Merck & Co., Inc., Genentech, the Mark Foundation, NIH-NIGMS, and NIH-NCI. C.R. and R.P. are employees and shareholders of F. Hoffmann-La Roche. M.J.M. reported the following potential competing interests: laboratory research and shareholder of F. Hoffmann-La Roche Ltd. clinical trials contracts for vaccines or MAB versus SARS-CoV-2 with Lilly, Pfizer, and Sanofi; personal fees for Scientific Advisory Board service from Merck, Meissa Vaccines, and Pfizer; contract funding from USG/HHS/BARDA for research specimen characterization and repository; research grant funding from USG/HHS/NIH for SARS-CoV-2 vaccine and MAB clinical trials. L.Z.R. served on the scientific advisory board for Biogen, Genentech, Celgene, and Novartis and received research support from Consortium of Multiple Sclerosis Centers; Biogen; and Genentech. M.C. is an employee and shareholder of Genentech, Inc. K.H. is a former employee of Genentech, Inc. J.P. is an employee of Genentech, Inc. and shareholder of F. Hoffmann-La Roche. T.E.B., R.C., Z.R., K.P., S.E., Y.Y., and A.S. have nothing to disclose.

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