Supplemental Online Content

Madelon N, Heikkilä N, Sabater Royo I, et al. Omicron-specific cytotoxic T-cell responses after a third dose of mRNA COVID-19 vaccine among patients with multiple sclerosis treated with ocrelizumab. *JAMA Neurol*. Published online February 25, 2022. doi:10.1001/jamaneurol.2022.0245

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods.

1.1. Study design

The study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. Adult individuals who were on anti-CD20 treatment (ocrelizumab) for MS and who received their third dose of mRNA COVID-19 vaccine before November 1st, 2021, were included in the herein performed analyses. Serum and peripheral blood mononuclear cells (PBMC) were collected on the day of vaccination and 30 days after the third dose of COVID-19 vaccine. Written informed consent was obtained from all participants.

1.2. Serology

Antibodies against epitopes from the vaccine-strain were measured in sera; anti-SARS-CoV-2 N (anti-N total antibodies) were detected on the Elecsys and anti-SARS-CoV-2 S (anti-RBD total antibodies) on the Cobas e801 analyzer (Roche Diagnostics, Switzerland). Seroconversion was defined as > 0.8 IU/ml for the anti-RBD antibodies and > 1 cut-off index (COI) for anti-N antibodies as previously defined[1].

1.3. T-cell assays

Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation and cryopreserved in FCS containing 10% DMSO in liquid nitrogen. Cryopreserved PBMC were thawed in complete RPMI supplemented with 10% FCS, essential amino acids and penicillin/streptomycin in the presence of benzonase (50 U/ml). Activation induced marker (AIM) assay was performed as described previously[1-3]. Briefly, cells were rested for 4 hours, resuspended at $20x10^6$ cells /ml in complete RPMI and stimulated overnight with 1 µg/ml of SARS-CoV-2 megapool peptides derived from the ancestral vaccine strain, Delta (B1.617.2) or Omicron strain (B1.1.529) in U-bottom 96 well plates at 37°C. SARS-CoV-2 megapool peptides were prepared as described previously[2]. In brief, 15-mer peptides overlapping by 10 amino acids predicted as CD4 or CD8 epitopes and spanning the entire spike protein for the ancestral strain, Delta and Omicron strains were synthesized separately as crude material, pooled and lyophilized. The lyophilized material was resuspended in DMSO at 1mg/ml so that each peptide is contained in the megapool. The mutations of the variant strains relative to the ancestral vaccine strain are shown in the table below. Stimulation with SEB at 1 µg/ml were included as positive controls. In addition, stimulation with an equimolar volume of DMSO was performed as negative control.

1.4. Mutations in the spike protein of Delta and Omicron variants relative to the ancestral vaccine-strain virus

Protein	Position	Vaccine strain	B.1.617.2 (Delta)	B.1.1. 529 (Omicron)
S	19	T	R	
S	67	A		V
S	69	H		del
S	70	V		del
S	95	T		I
S	142	G		D
S	143	V		del
S	144	Y		del
S	145	Y		del
S	152	W		del
S	211	N		del
S	212	L		I
S	214	ins		EPE
S	339	G		D
S	371	S		L
S	373	S		P
S	375	S		F
S	417	K		N
S	440	N		K
S	446	G		S
S	452	L	R	
S	477	S		N
S	478	T	K	K
S	484	E		A
S	493	Q		R
S	496	G		S
S	498	Q		R
S	501	N		Y
S	547	T		K
S	614	D	G	G
S	655	H		Y
S	679	N		K
S	681	P	R	H
S	764	N		K
S	796	D		Y
S	856	N		K
S	950	D	N	
S	954	Q		H
S	969	N		K
S	981	L		F

1.5. Flow cytometry

Cells were surface stained 20 minutes at 4°C for activation induced markers, and fixed/permeabilized with the FoxP3 transcription factor buffer kit (Thermofischer scientific) for 30 minutes at 4°C. Stained cells were analysed by flow cytometry on FACS Fortessa (BD) and data analysis was performed using FlowJo software (version 10, Treestar). For dead cell exclusion, LIVE/DEAD fixable Aqua kit was used (ThermoFischer). All antibodies were purchased from Biolegend or BD Bioscience (see table). Data presented were background subtracted. The cut-off limit for responders in the activation induced (AIM) assay was 0.05%. The cut-off was determined after calculating the mean frequencies of AIM+ CD4 and CD8 T cells after DMSO stimulation in 58 samples[1] (CD4 T cells, mean 0.046%, IQR 0.008 – 0.05, CD8 T cells 0.033%, IQR 0.005 – 0.019) and given the heterogeneity rounded to the upper 2nd digit.

1.6. Fluorescent-labelled antibodies used in flow cytometric assay

Antibodies	**Fluorochrome**	**Clone**	**Brand**	**Reference**
CD3	A700	SK7	Biolegend	344822
CD4	APC-H7	RPA-T4	BD	560158
CD8	BUV737	SK1	BD	612754
CD45RA	BV785	HI100	Biolegend	304140
CCR7	PE-Dazzle 594	G043H7	Biolegend	353236
CD69	PerCP-Cy5.5	FN50	BD	560738
OX40	BUV395	ACT35	BD	743286
4-1BB	APC	4B4-1	Biolegend	309810

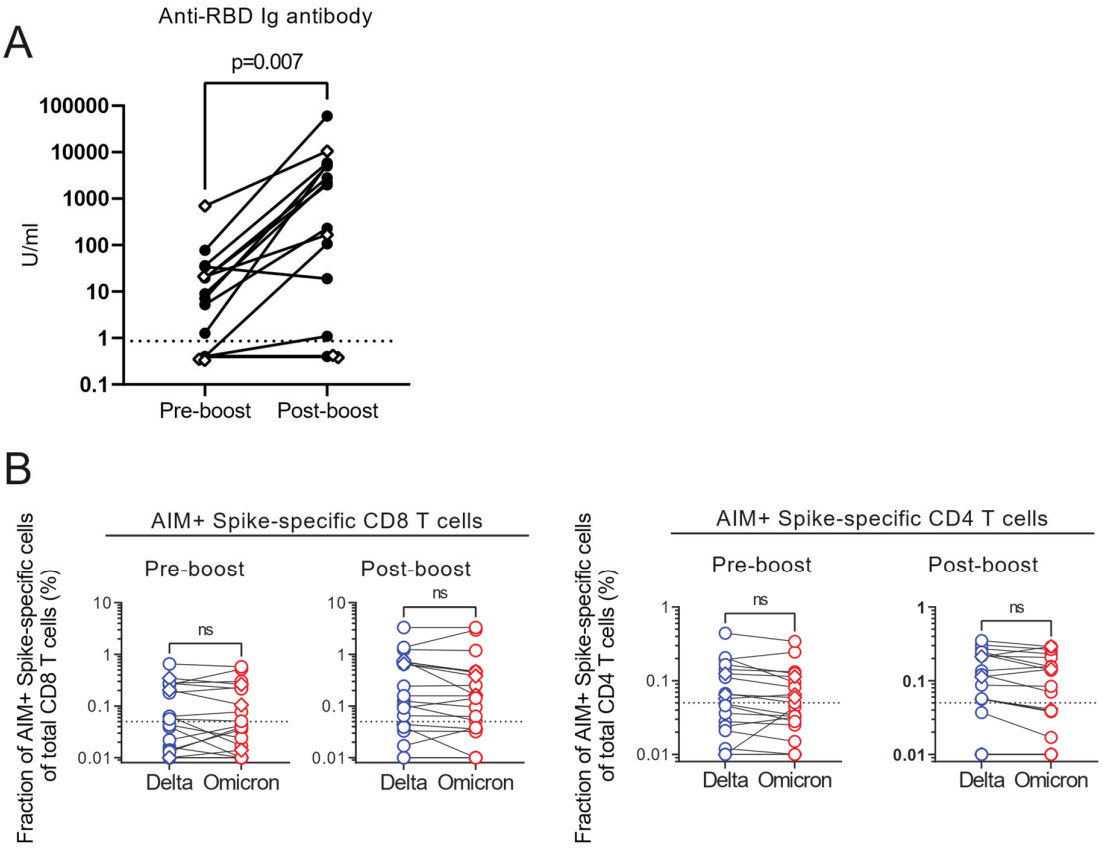
1.7. Statistics

Statistical analysis was performed with GraphPad Prism software (version 8.0.2). Quantitative variables were compared by Wilcoxon signed rank test either including the entire cohort or responders only, as indicated, and p values < 0.05 (2-tailed) were considered statistically significant.

1.8. References

1. Madelon N, Lauper K, Breville G, et al. Robust T cell responses in anti-CD20 treated patients following COVID-19 vaccination: a prospective cohort study. *Clin Infect Dis.* 2021.
2. Grifoni A, Weiskopf D, Ramirez SI, et al. Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. *Cell.* 2020;181(7):1489-1501 e1415.
3. Tarke A, Sidney J, Methot N, et al. Impact of SARS-CoV-2 variants on the total CD4(+) and CD8(+) T cell reactivity in infected or vaccinated individuals. *Cell reports Medicine.* 2021;2(7):100355.

eFigure. Comparison of anti-RBD antibody and frequencies of Delta- and Omicron-specific T cells



(A) Anti-RBD antibody levels are indicated before and one month after the third vaccine dose. Individual patient results are connected by a line, and patients vaccinated with mRNA-1273 are depicted as closed circles and those with BNT162b2 as open diamonds. (B) Graphs showing differences in frequencies of CD8 (left) and CD4 (right) T cells specific for the spike protein of the Delta vs. Omicron variant in patients before (left) and 30 days after (right) the third vaccine dose. Wilcoxon signed ranks test was used to assess differences before and after the third dose (A+B).

eTable. Effector memory phenotype of spike-specific CD4 and CD8 T cells in anti-CD20–treated MS patients before and after the third vaccine dose

	CD4 AIM+		CD4 AIM-		CD8 AIM+		CD8 AIM-	
	Pre-boost	**Post-boost**	**Pre-boost**	**Post-boost**	**Pre-boost**	**Post-boost**	**Pre-boost**	**Post-boost**
TNAIVE	3,42 (2,51-7,01) 4,87 (2,29-7,52) 4,20 (2,33-7,87)	3,29 (1,71-4,08) 2,91 (1,36-5,87) 3,60 (1,60-6,31)	40,05 (34,70-51,78) 40,90 (36,68-51,63) 41,80 (36,13-51,78)	41,30 (33,68-53,65) 41,80 (36,05-53,93) 42,40 (36,20-52,98)	7,60 (2,98-15,55) 6,61 (5,09-16,68) 10,20 (6,05-13,75)	3,920 (2,58-13,80) 4,09 (2,42-11,05) 5,85 (2,30-14,13)	22,95 (11,70-36,50) 23,70 (13,40-35,98) 24,30 (13,20-38,80)	20,00 (14,43-40,68) 20,35 (15,08-41,85) 21,80 (15,73-41,88)
TCM	41,50 (37,60-51,70) 45,30 (37,03-50,45) 43,30 (36,05-52,80)	39,50 (33,10-53,40) 38,35 (33,58-44,93) 34,90 (28,80-43,20)	34,55 (26,85-41,25) 33,50 (27,10-40,23) 32,65 (26,63-40,48)	36,65 (27,00-42,05) 36,35 (26,35-41,48) 36,40 (26,33-40,43)	16,15 (13,03-27,18) 15,85 (11,10-31,83) 17,20 (15,90-29,65)	18,10 (11,40-23,80) 15,75 (6,52-21,85) 14,75 (11,75-18,53)	15,10 (8,39-24,10) 15,50 (8,09-24,00) 14,55 (7,82-20,65)	15,30 (9,08-24,30) 15,75 (9,96-23,03) 14,75 (9,15-19,90)
TEM	42,30 (36,10-55,35) 45,75 (32,58-56,78) 40,70 (36,53-55,68)	52,90 (39,70-59,50) 53,05 (43,83-62,35) 55,30 (51,90-59,70)	17,10 (15,55-22,80) 16,85 (14,75-24,35) 17,85 (14,83-21,78)	16,30 (14,18-21,93) 16,50 (13,98-20,50) 16,40 (14,00-22,08)	42,25 (36,65-55,25) 49,05 (37,78-57,10) 50,00 (32,05-60,45)	51,90 (38,80-67,80) 53,45 (41,10-71,48) 58,75 (36,25-66,18)	32,55 (24,83-39,25) 34,25 (24,48-41,80) 32,55 (24,23-37,48)	32,20 (20,88-46,10) 30,75 (21,08-45,70) 31,15 (20,90-44,10)
TEMRA	4,74 (1,25-8,76) 3,44 (1,75-8,40) 5,16 (1,83-7,37)	2,79 (1,29-4,47) 3,88 (2,02-5,15) 2,06 (1,01-5,70)	1,83 (0,70-4,56) 1,83 (0,64-4,54) 1,98 (0,69-4,99)	1,81 (0,68-4,16) 1,76 (0,70-3,88) 1,81 (0,70-3,81)	25,45 (15,83-35,85) 23,25 (9,92-34,23) 13,50 (9,10-30,90)	18,60 (11,40-29,50) 22,10 (14,70-31,80) 19,15 (15,95-30,53)	15,55 (12,18-30,40) 16,30 (11,88-31,15) 16,85 (12,83-31,43)	18,35 (10,95-31,93) 19,05 (11,40-33,03) 18,65 (11,50-32,35)

Table describing the memory phenotype of Spike-specific CD4 (CD4 AIM+) T cells of individuals with detectable AIM+ CD4 T cells for SARS-CoV-2 vaccine strain (depicted in dark, n=9 pre-boost, 15 post-boost), and variants Delta (in blue, n=10 pre-boost, n=14 post boost) and Omicron (in red, n=10 pre-boost, n=11 post-boost). The median frequency + IQR of naïve (T_{NAIVE}, $CD45RA^+$ $CCR7^+$), central memory (T_{CM}, $CD45RA^-$ $CCR7^+$), effector memory (T_{EM}, $CD45RA^-$ $CCR7^-$) and RA-positive effector memory T cells (T_{EMRA}, $CD45RA^+$ $CCR7^-$) are shown.

The proportions of memory phenotype Spike-specific AIM+ CD8 T cells of individuals with detectable AIM+ CD8 T cells for SARS-CoV-2 vaccine strain (n=12 pre-boost, n=15 post boost), and variants Delta (n=10 pre-boost, n=14 post boost) and Omicron (n=9 pre-boost, n=14 post-boost) are shown.

Results of bulk non-specific CD4 (CD4 AIM-) and CD8 T cells (CD8 AIM-) are shown (n=20).