

Methotrexate Hampers Immunogenicity to BNT162b2 mRNA COVID-19 Vaccine in Immune-Mediated Inflammatory Disease

Supplementary Appendix

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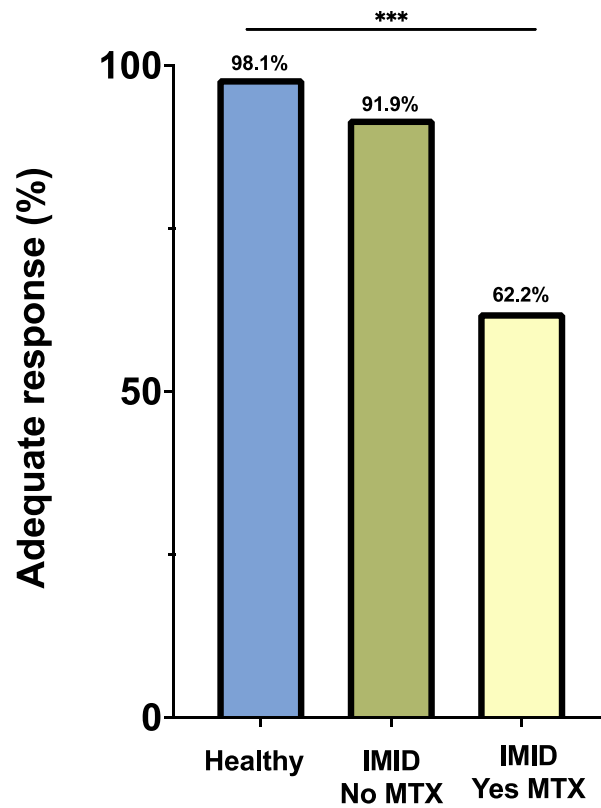
Supplementary Table 1. Characteristics of cohort from Erlangen, Germany.

Characteristic	Controls (n = 182)	IMID No MTX ⁺ (n = 11)	IMID Yes MTX ⁺ (n = 20)	p-value
Age- mean (range, SD)	40.8 [21-65, 12.0]	45.0 [26-80, 15.5]	54.5 [24-87, 19.2]	0.008
Female- n (%)	104 (57.1)	7 (58.3)	15 (75.0)	0.290
Race- n (%)				
White	178 (97.8)	11 (100.0)	19 (95.0)	0.680
Black	0 (0.0)	0 (0.0)	0 (0.0)	
Asian	2 (1.1)	0 (0.0)	1 (5.0)	
Other	1 (0.6)	0 (0.0)	0 (0.0)	
Hispanic ethnicity- n (%)	1 (0.6)	0 (0.0)	0 (0.0)	0.918
Long term medication use- n (%)				
Methotrexate	--	0 (0.0)	20 (100.0)	1.00
Tumor necrosis factor inhibitors	--	11 (100.0)	0 (0.0)	1.00
Methotrexate dose- mean (SD)	--	--	14.7 (4.2)	
COVID-19 infection prior to vaccination- n(%)	0 (0.0)	0 (0.0)	0 (0.0)	1.00
Days from 1 st vaccine mean (range, SD)	47.7 (11-78,12.1)	42.1 (23-74,18.0)	44.0 (13-78,16.7)	0.204
Number receiving 2 nd vaccination dose- n (%)	179 (98.4)	9 (81.8)	18 (90.0)	0.044
Adequate humoral immunogenicity [#] - n (%)	179 (98.4)	10 (90.9)	10 (50.0)	<0.001
OD anti-SARS-CoV-2 IgG				
Median (range)	9.4 (1.2-14)	7.8 (2.3-11.3)	5.9 (0.95-13.5)	<0.001

⁺IMID denotes immune-mediated inflammatory disease, MTX methotrexate, OD optical density.

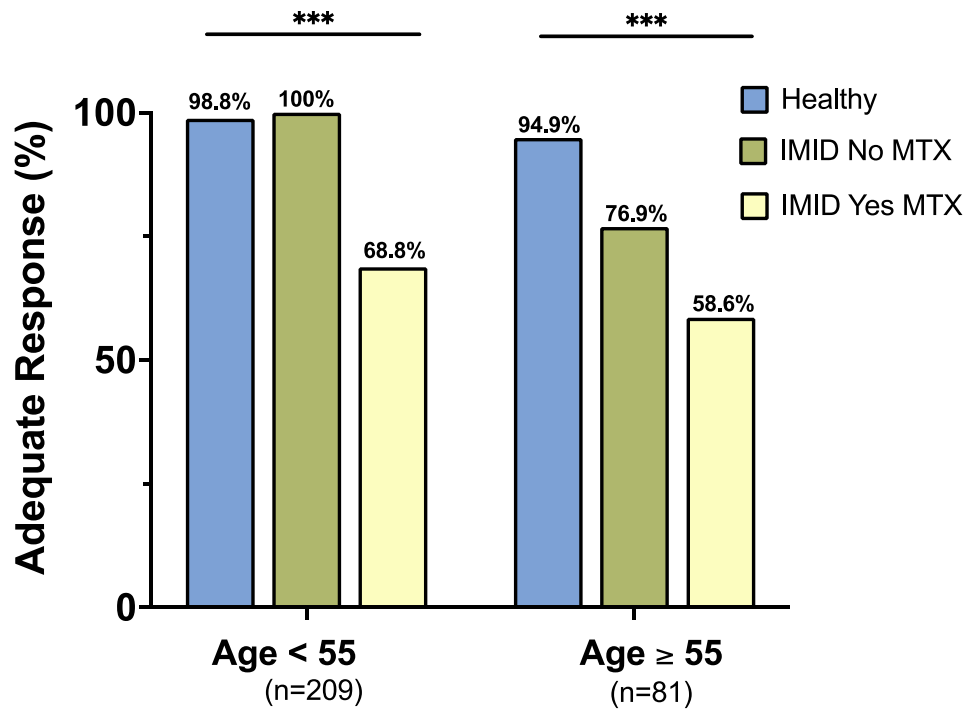
^{*}Giant cell arteritis and polymyalgia rheumatica.

[#]Adequate response defined as within 2 standard deviations (SD) of the mean OD450nm of controls (OD 450nm >5.7units).

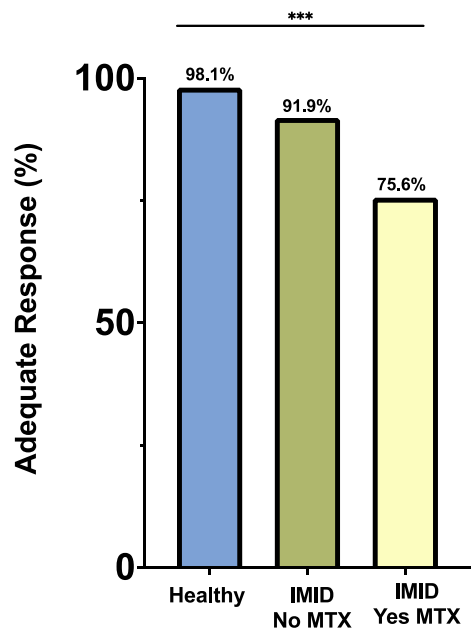


Supplementary Figure 1. Percentage of patients achieving adequate response to COVID-19 vaccination from New York and Erlangen cohorts combined (n=290). Percentages and group comparisons using chi squared test of independence reflect proportion of those achieving an adequate response within each group.

***indicated p value less than .001.



Supplementary Figure 2. Percentage of total patients achieving adequate response to COVID-19 vaccination by age. Percentages and group comparisons using chi squared test of independence reflect proportion of those achieving an adequate response within each group. ***indicated p value less than .001.



Supplementary Figure 3. Percentage of total patients achieving a stricter definition of adequate response to COVID-19 vaccination. For the New York City cohort, adequate response is defined as greater than 1000 units and for the Erlangen cohort, adequate response is defined as greater than 5.0 (OD450nm). Percentages and group comparisons using chi squared test of independence reflect proportion of those achieving an adequate response within each group. ***indicated p value less than .001.

Supplementary Methods

Anti-SARS-CoV-2 IgG antibody titers

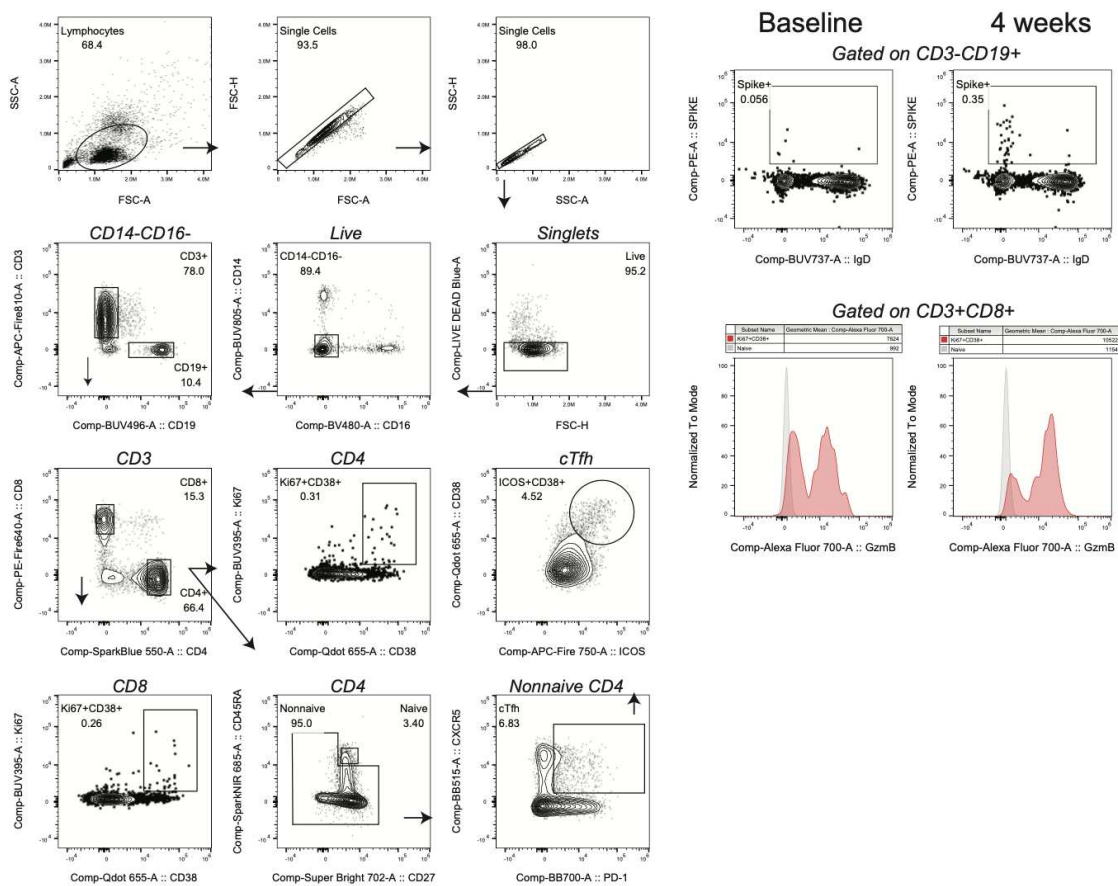
Direct ELISA was used to quantify antibody titers in the New York cohort participants serum. Ninety-six well plates were coated with 1 µg/ml S1 protein (100 µl/well) diluted in PBS and were then incubated overnight at 4°C (Sino Biological Inc., 40591-V08H). Plates were washed four times with 250 µl of PBS containing 0.05% Tween 20 (PBS-T) and blocked with 200 µl PBS-T containing 4% non-fat milk and 5% whey, as blocking buffer at RT for 1 hour. Sera were heated at 56°C for 1 hour prior to use. Samples were diluted to a starting concentration of 1:50 (S1) were first added to the plates and then serially diluted 1:3 in blocking solution. The final volume in all wells after dilution was 100 µl. After a 2-hour incubation period at RT, plates were washed four times with PBS-T. Horseradish-peroxidase conjugated goat-anti human IgG (Southern BioTech, 2040-05) were diluted in blocking buffer (1:2000) and 100 µl was added to each well. Plates were incubated for 1 hour at RT and washed four times with PBS-T. After developing for 5 min with TMB Peroxidase Substrate 3,3',5,5'-Tetramethylbenzidine (Thermo Scientific), the reaction was stopped with 1M sulfuric acid or 1N hydrochloric acid. The optical density was determined by measuring the absorbance at 450 nm on a Synergy 4 (BioTek) plate reader. In order to summarize data collected on individuals, the area under the response curve was calculated for each participant and end point titers were normalized using replicates of pooled positive control sera on each plate to reduce variability between plates. IgG antibodies against the S1 domain of the spike protein of SARS-CoV-2 were tested in the Erlangen participants using the commercial enzyme-linked immunosorbent assay from Euroimmun (Lübeck, Germany) on the EUROIMMUN Analyzer I platform and according to the manufacturers protocol. Optical density was determined at 450 nm with reference wavelength at 630 nm.

Immune cell phenotyping by high-dimensional spectral flow cytometry

Peripheral blood was collected in sodium heparin collection tubes and PBMC were isolated using the SepMate system in accordance with manufacturer's instructions. PBMC were cryopreserved in fetal calf serum supplemented with 10% DMSO. Cryopreserved cells were thawed in batches for immunophenotyping studies. Then, 2 to 5 million freshly isolated PBMC were resuspended in HBSS supplemented with 1% fetal calf serum (Fisher) and 0.02% sodium azide (Sigma). Cells underwent Fc-blockade with Human TruStain FcX (Biolegend) and NovaBlock (Phitonex) for 10 minutes at room temperature, followed by surface staining antibody cocktail at room temperature for 20 minutes in the dark. Cells were permeabilized with the eBioscience Intracellular Fixation and Permeabilization kit (Fisher) for 20 minutes at room temperature in the dark, followed by intracellular staining with an antibody cocktail for 1 hour at room temperature in the dark. All samples were then resuspended in 1% paraformaldehyde and acquired within three days of staining on a 5-laser Aurora cytometer (Cytex Biosciences). Antibodies, clones, and catalog numbers are available in Table S3. Initial data quality control was performed using FlowJo.

Supplementary Table 2. Antibodies used for high-dimensional spectral flow cytometry

Target	Fluorochrome	Clone	Manufacturer	Catalog #
Live/Dead Blue	-	-	Invitrogen	L23105
CD3	APC/Fire 810	SK7	Biologend	344857
CD4	SparkBlue 550	SK3	Biologend	344656
CD8	PE-Fire 640	SK1	Biologend	344761
CD11c	PerCP	Bu15	Biologend	337234
CD14	BUV805	M5E2	BD	612902
CD16	BV480	3G8	BD	566171
CD19	BUV496	SJ25C1	BD	612939
CD20	APC	2H7	Biologend	302309
CD21	PE-Cy5	B-ly4	BD	551064
CD23	BUV615	M-L233	BD	751104
CD25	BUV563	2A3	BD	612919
CD27	SB702	O323	Invitrogen	67-0279-42
CD38	Qdot655	HIT2	Invitrogen	Q22150
CD40	BV510	5C3	Biologend	334330
CD45RA	Spark NIR 685	HI100	Biologend	304168
CD56	BV570	5.1H11	Biologend	362539
CD71	SB780	OKT9	Invitrogen	78-0719-42
CD123	BV650	7G3	BD	563405
Recombinant Spike protein	PE		Biologend	793804
CD138	PacBlue	MI15	Biologend	356531
CD150 (CTLA4)	BV421	BNI3	Biologend	369606
CD183 (CXCR3)	BV750	1C6	BD	746895
CD185 (CXCR5)	BB515	RF8B2	BD	564624
CD197 (CCR7)	BV605	G043H7	Biologend	353224
CD278 (ICOS)	APC-Fire750	C398.4A	Biologend	313536
CD279 (PD-1)	BB700	EH12.1	BD	566460
HLA-DR	BUV661	G46-6	BD	612980
IgD	BUV737	IA6-2	BD	612798
Foxp3	PE-Cy5.5	PCH101	Invitrogen	35-4776-42
Tbet	PE-Cy7	4B10	Biologend	644823
Eomes	PE-eF610	WD1928	Invitrogen	61-4877-42
GzmB	A700	GB11	BD	561016
Ki67	BUV395	B56	BD	564071
IgG	PerCP-Vio700	IS11-3B2.2.3	Miltenyi	130-119-880



Supplementary Figure 4. Flow cytometry gating strategy. (Left panel) Gating strategy and example plots for the key immune populations. (Right panel) Plots showing baseline or at the 4-week time point for CD19 specific for Spike protein (top row) or for expression of Granzyme B in CD8 T cells