SUPPLEMENTARY DATA

Interaction between B-cell activation factor and methotrexate impacts immunogenicity of seasonal influenza vaccination in patients with rheumatoid arthritis.

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SUPPLMENTARY METHODS

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SUPPLMENTARY METHODS

Patients and vaccination.

Patients from the prospective multicenter randomized investigator-blind parallel-group intervention study that aimed to investigate the effects of a two-week MTX discontinuation on vaccine response to 2016-17 seasonal influenza vaccination in patients with RA were included [9]. As previously described, patients with RA according to the revised 1987 American College of Rheumatology, who were aged 19 years or older and had been on the stable dose of MTX for six weeks or longer, were randomized to continue MTX or to hold it for two weeks after vaccination with 2016-17 seasonal quadrivalent influenza vaccine (GC Flu™, GC Pharma, South Korea) that contained four antigens: 15 μg of A/California/7/2009 Reassortant virus NYMC X-181 (H1N1), 15 μg of A/Hong Kong/4801/2014 NYMC X-263B (H3N2), 15 μg of B/Phuket/3073/2013 (B-Yamagata), and 15 μg of B/Brisbane/60/2008 in a 0.5-mL prefilled syringe. Patients, in whom serum at the time of vaccination was not available for BAFF measurement, were excluded.

BAFF measurement

Patient serum before the vaccination (i.e. visit 1) was stored at -20 °C to -80 °C until assay. Human BAFF/BLys/TNFSF13B Immunoassay for BAFF was performed according to the manufacturer's instruction (R&D Systems). Specific absorbance was measured at 450 nm and 560 nm, and optical density was quantified by sunrise™ absorbance microplate reader (Tecan).

Vaccine response

Before (week 0) and at four weeks after vaccination, the hemagglutination inhibition (HI) antibody titers against each of the four influenza strains were measured as previously described. A positive vaccine response was defined as a ≥4-fold increase in HI antibody titer at four weeks after vaccination relative to the baseline.

Statistical analysis

Continuous variables were analyzed by using a t-test. The binary secondary efficacy variables were analyzed by using chi-squared tests or Fisher's exact test, as appropriate. Correlation of BAFF levels with RA characteristics or HI antibody titer changes were investigated using Spearman's or Pearson's correlation as appropriate. Linear regression model was used to estimate the impact BAFF levels on vaccine response (i.e. antibody fold change after vaccination) after adjusting for use of biologics and corticosteroid dose in the MTX continue and the MTX-hold group. To analyze the impact of MTX-BAFF interaction on antibody formation, a general linear model was used with log-transformed antibody fold change as a dependent variable, MTX-use as a fixed variable and BAFF levels as a covariate.

$P<0.05$ was considered to indicate statistical significance. All analyses were performed by using SPSS (IBM SPSS statistics version 22).

Supplementary Figure S1. Patient flow.
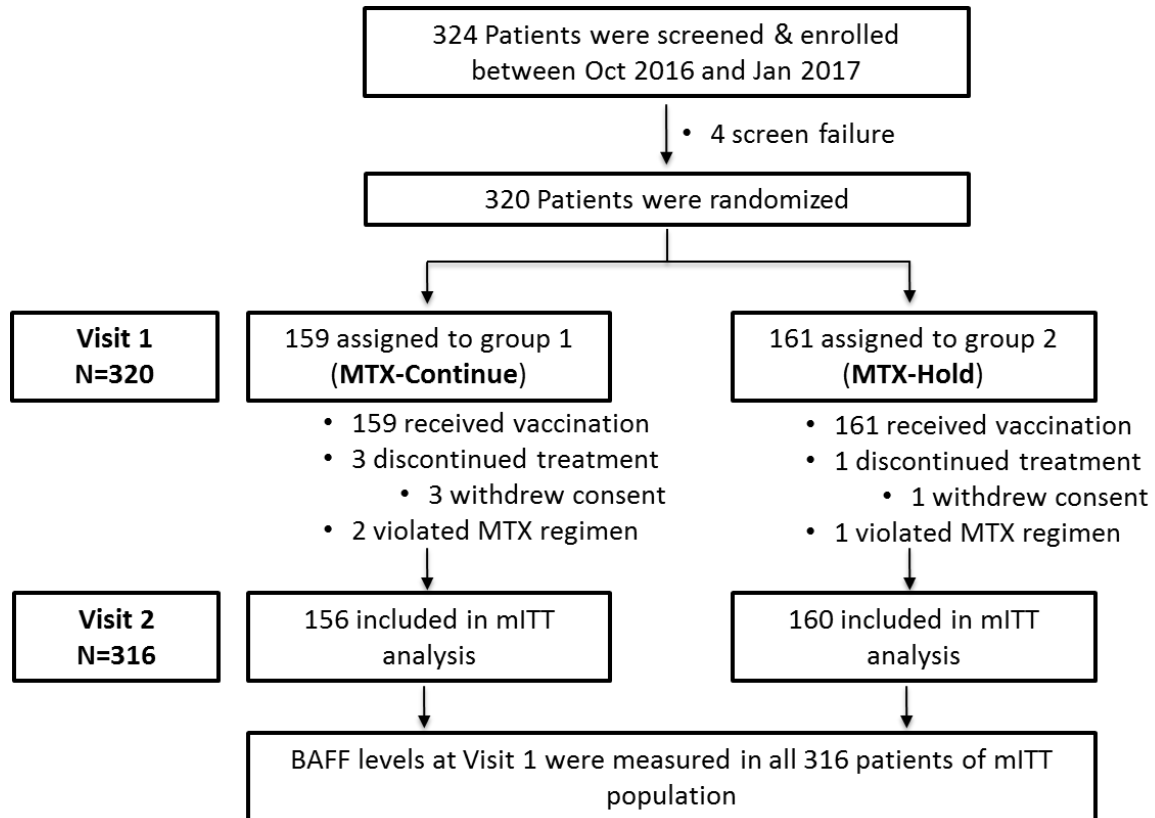
Supplementary Table S1. Correlation coefficients (rho) and p values between the baseline BAFF levels and RA characteristics in RA patients. Correlation was examined by using Spearman correlation.

		Age	Dis	DAS28	WBC	ALC	CRP	RF-titer	ACPA-titer	PD-dose	MTX-dose
BAFF	**r**	0.234	0.088	0.020	-0.089	-0.211	0.079	-0.013	-0.009	-0.177	0.010
	p	**<0.001**	0.119	0.730	0.115	**<0.001**	0.162	0.813	0.890	**0.002**	0.856
	N	316	316	316	316	288	316	309	258	316	316

ALC, RF, ACPA titer were not available in all patients.

ACPA=Anti-cyclic citrullinated peptide-antibody; ALC=absolute lymphocyte count; BAFF=B-cell activation factor; CRP=C-reactive protein; DAS28=Disease Activity Score in 28 joints; Dis=disease duration; GC=glucocorticoids; MTX=methotrexate; r=rho; RA=rheumatoid arthritis; RF=rheumatoid factor; WBC=white blood cell count.

Supplementary Table S2. Correlation between BAFF levels and antibody fold change by MTX-dose in the MTX continue group.

MTX (mg/wk)	N		Spearman	H1N1	H3N2	Yamagata	Victoria
<7.5	18	**BAFF**	rho	0.080	0.086	-0.288	-0.120
			p	0.753	0.736	0.247	0.634
10-12.5	40	**BAFF**	rho	-0.154	-0.106	-0.214	-0.281
			p	0.341	0.515	0.184	0.079
>15	98	**BAFF**	rho	-0.256	-0.058	-0.235	-0.216
			p	**0.011**	0.573	**0.020**	**0.033**

Correlation was examined using Spearman correlation according to weakly MTX dosage. MTX=methotrexate. Numbers in bold are statistically significant.

Supplementary Table S3. Association of BAFF levels, daily corticosteroid dose, and use of biologics in the MTX continue group and the MTX hold group.

		MTX continue					MTX hold				
		B	Std. Error	Beta	T	Sig	B	Std. Error	Beta	T	Sig
H1N1	**BAFF**	-0.001	0.000	-0.162	-1.993	0.048	0.000	0.000	0.032	0.404	0.687
	PD	0.059	0.052	0.090	1.128	0.261	0.087	0.054	0.128	1.621	0.107
	Biol	-0.291	0.382	-0.062	-0.764	0.446	-0.399	0.364	-0.087	-1.097	0.274
H3N2	**BAFF**	0.000	0.000	-0.090	-1.092	0.277	0.000	0.000	0.051	0.633	0.528
	PD	-0.042	0.050	-0.068	-0.841	0.402	-0.020	0.053	-0.031	-0.383	0.702
	Biol	-0.324	0.368	-0.072	-0.881	0.380	-0.295	0.358	-0.066	-0.823	0.412
Yamagata	**BAFF**	-0.001	0.000	-0.207	-2.544	0.012	0.000	0.000	0.028	0.347	0.729
	PD	0.028	0.046	0.048	0.607	0.545	0.045	0.042	0.086	1.084	0.280
	Biol	0.058	0.334	0.014	0.173	0.863	-0.457	0.284	-0.127	-1.608	0.110
Victoria	**BAFF**	0.000	0.000	-0.150	-1.835	0.068	0.000	0.000	0.028	0.347	0.729
	PD	0.042	0.041	0.080	1.002	0.318	0.045	0.042	0.086	1.084	0.280
	Biol	-0.051	0.304	-0.014	-0.166	0.868	-0.457	0.284	-0.127	-1.608	0.110

Linear regression was performed using the log-transformed antibody fold changes as independent variables and BAFF levels, daily PD dose (mg/day) and use of biologics as independent variables.

Biologics include tumor necrosis factor inhibitors (n=11), tocilizumab (n=4), abatacept (n=1), and rituximab (n=1). BAFF=B-cell activation factor; Biol=biologic DMARDs; MTX=methotrexate; PD=prednisolone (in mg/week).