

Alterations in B Cell Subsets Correlate with Body Composition Parameters in Female Adolescents with Anorexia Nervosa

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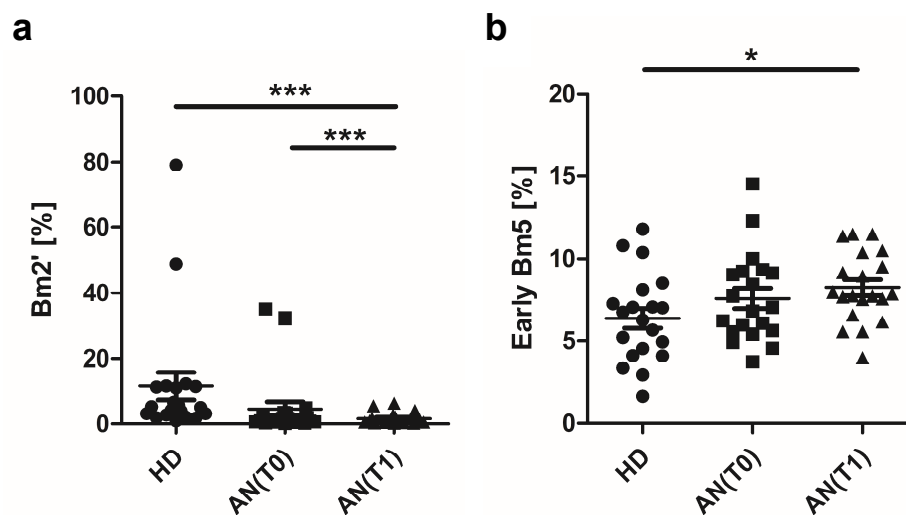


Figure S1. Mature B cell (Bm) subpopulations in PBMC of healthy controls (HC) and adolescents with anorexia nervosa (AN). Graphs displaying frequencies of (a) Bm2' cells and (b) early Bm5 cells in HC (n=20) and AN at T0 (n=20) and at T1 (n=20). *P*-values vs. HC were calculated by *t* test for independent samples, T0 vs. T1 by *t* test for paired samples, **p*<0.05; ****p*<0.001.

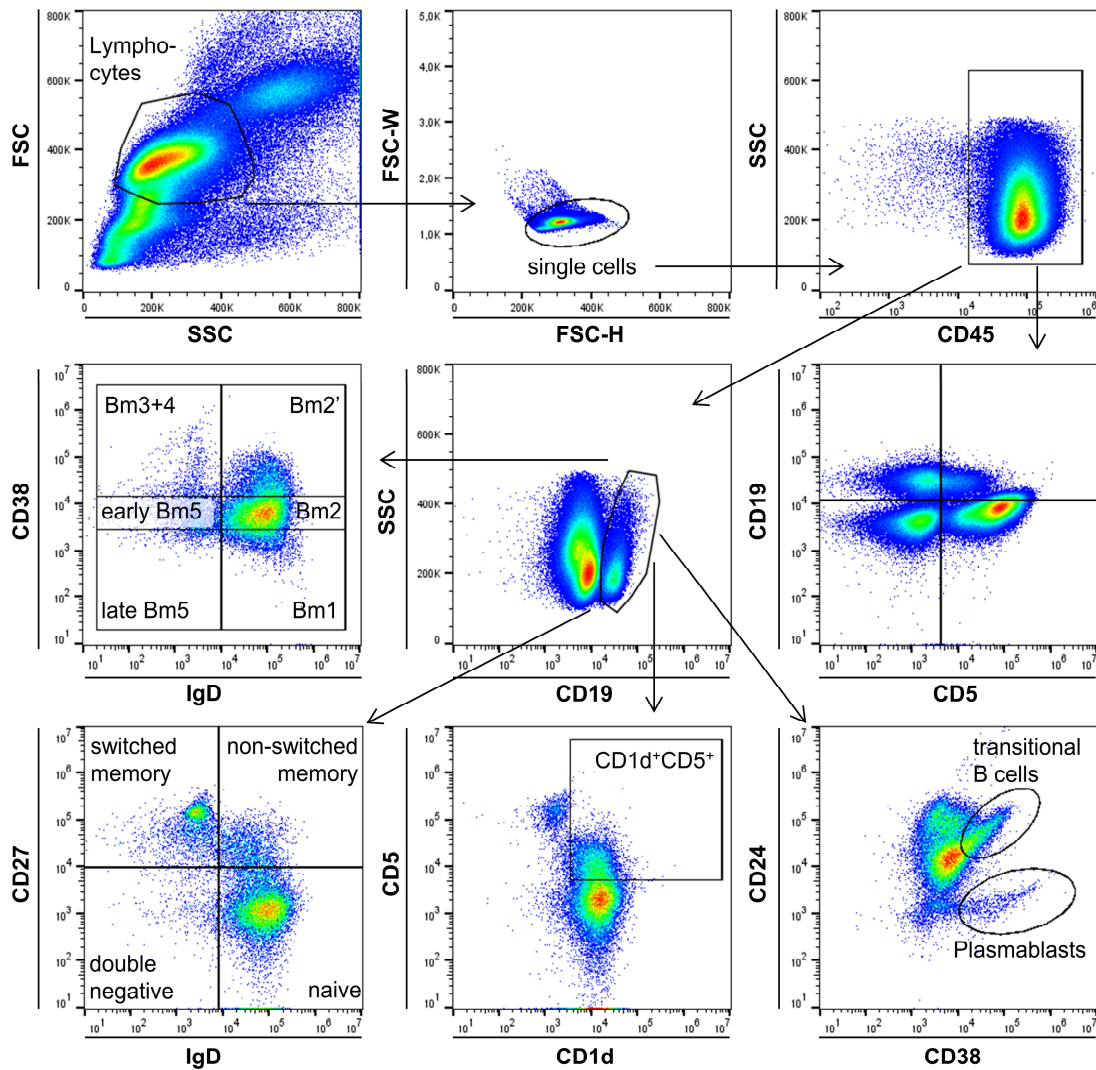


Figure S2. Exemplary gating strategy for phenotypic characterization of B cell subsets in adolescents with anorexia nervosa (AN) and healthy controls (HC). Lymphocytes from peripheral blood mononuclear cells (PBMC) were selected from total events based on their size (forward scatter [FSC]) and granularity (side scatter [SSC]). Single cells were gated based on FSC-Width vs FSC-Height information. Pre-gated on CD45⁺ and CD19⁺, cells were further gated for transitional B cells (CD24^{high}CD38⁺) and plasmablasts (CD24^{low}CD38⁺), for CD1d⁺CD5⁺ regulatory B cells, as well as for naive (IgD⁺CD27⁻), switched memory (IgD⁻CD27⁺), and non-switched memory B cells (IgD⁺CD27⁺). Additionally, B cells were subdivided into Bm1, Bm2, Bm2', Bm3+4 and early and late Bm5 cells based on CD38 vs. IgD expression.

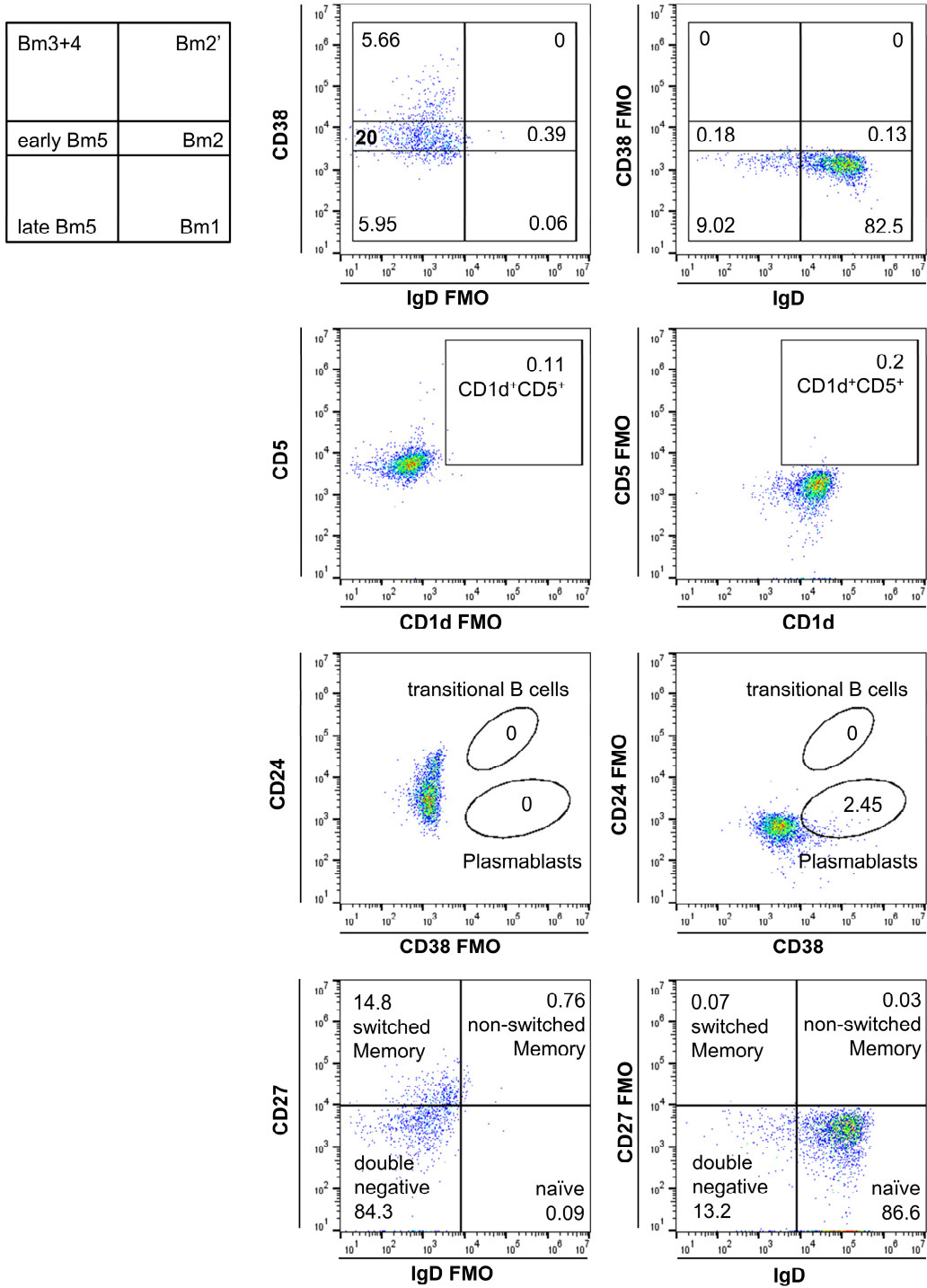


Figure S3. Representative fluorescence minus one (FMO) controls for the corresponding gating strategy.

Table S1. Clinical characteristics of patients with anorexia nervosa (AN) at T0.

	AN (T0)
	(n=24)
Premorbid BMI (kg/m ² , mean ± SD)	20.4 ± 2.8 ⁺
Premorbid BMI percentile (mean ± SD)	45.2 ± 28.3 ⁺
Premorbid BMI SDS (mean ± SD)	-0.1 ± 0.9 ⁺
Inpatient pretreatment (yes/no, n)	5/19

⁺n=23; BMI: body mass index; SDS: standard deviation score.

Table S2. Correlation coefficients of immune parameters with clinical characteristics for HC, AN(T0) or AN(T1).

	HC (n=20)						AN(T0), (n=24)						AN(T1), (n=20)					
	BMI SDS (KIGGS)	BMI percentile	BMI [kg/m ²]	FM [%] (n=19)	FMI [kg/m ²] (n=19)	FFMI [kg/m ²] (n=19)	BMI SDS (KIGGS)	BMI percentile	BMI [kg/m ²]	FM [%] (n=23)	FMI [kg/m ²] (n=23)	FFMI [kg/m ²] (n=23)	BMI SDS (KIGGS)	BMI percentile	BMI [kg/m ²]	FM [%]	FMI [kg/m ²]	FFMI [kg/m ²]
Lymphocytes	.121	.135	-.065	-.460*	-.396	.325	-.268	-.268	-.239	-.001	.061	-.276	.046	-.093	.148	.052	.008	.082
CD19 ⁺ B cells	-.218	-.262	-.107	.102	.048	-.211	.122	.122	.123	.017	.057	.296	.362	.362	.163	.159	.178	.017
Naive B cells	-.037	.032	-.101	.301	.219	-.405	.274	.274	.164	.105	.175	-.087	.211	.285	.067	.183	.134	-.080
Non-switched memory B cells	-.002	-.067	.128	-.144	-.095	.288	-.297	-.297	-.347	.223	.072	-.311	-.218	-.136	-.001	.299	.312	-.240
Switched memory B cells	.113	.036	.147	-.244	-.154	.402	-.367	-.367	-.125	-.197	-.172	.099	-.268	-.283	-.055	-.174	-.163	.079
Plasmablasts	.245	.245	.338	.225	.405	.180	-.029	-.029	-.161	-.208	-.247	-.202	-.120	-.120	-.220	.047	.120	-.099
Transitional B cells	-.262	-.262	-.087	.047	.093	-.211	.119	.119	.050	.230	.205	-.100	.435	.435	.305	-.012	.021	.477*
Late Bm5 cells	.173	.198	.118	-.121	-.026	.226	-.175	-.175	.041	.066	.128	.181	-.340	-.444	-.112	.143	.144	-.194
CD1d ⁺ CD5 ⁺ B cells	.037	.067	.082	.171	.155	-.061	.213	.213	-.087	.456*	.474*	-.003	.485*	.421	.178	.253	.288	-.052
CD5 MFI on transitional B cells	.200	.243	.097	.032	.081	.056	.190	.190	.165	.438*	.428*	-.052	.405	.168	.159	.557*	.568**	-.303

#n=19, *n=23; HC: healthy control; AN: Anorexia nervosa; BMI: body mass index; SDS: standard deviation score; KIGGS: German Health Interview and Examination Survey for Children and Adolescents; FM: fat mass; FMI: fat mass index; FFMI: fat free mass index; italics show Spearman Rho correlation coefficients, otherwise correlation is given according to Pearson; bold printed values mark significant correlations; * $p < .05$; ** $p < .01$.

Table S3. Contraceptive, supplements and medication of patients with AN at T0 and T1.

	AN(T0)	AN(T1)
Oral contraceptive	3	3
Supplements		
Vitamin D	3	9
Nutritional drink (Fresubin)	2	2
Sodium glycerophosphate	1	0
Magnesium	0	2
Benzodiazepine	1	0
Phenothiazine (Promethazine)	0	1
Domperidone	1	1
SSRI	0	1
Antipsychotic drug (Quetiapine, low dose)	0	2
Antibiotics (Flucloxacillin)	0	1

Table S4. Antibodies used for extracellular staining.

Specificity	Fluorochrome	Clone
CD1d	PE	51.1
CD5	APC	UCHT2
CD19	PerCP	HIB19
CD24	BV510	ML5
CD27	PE-Cy7	M-T271
CD38	BV421	HIT2
CD45	APC-Cy7	HI30
IgD	FITC	IA6-2