

Supplementary file

Peripheral blood lymphocyte phenotype differentiates secondary antibody deficiency in rheumatic disease from primary antibody deficiency

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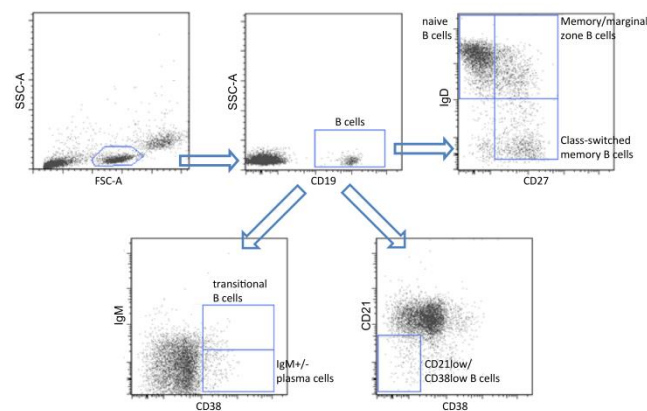


Figure S1. Strategy to measure B cell subsets. After doublet exclusion (not shown) and gating on lymphocytes based on SSC and FSC, we selected CD19⁺ B cells with subsequent gating based on IgD, IgM, CD27 and CD38 expression, as indicated.

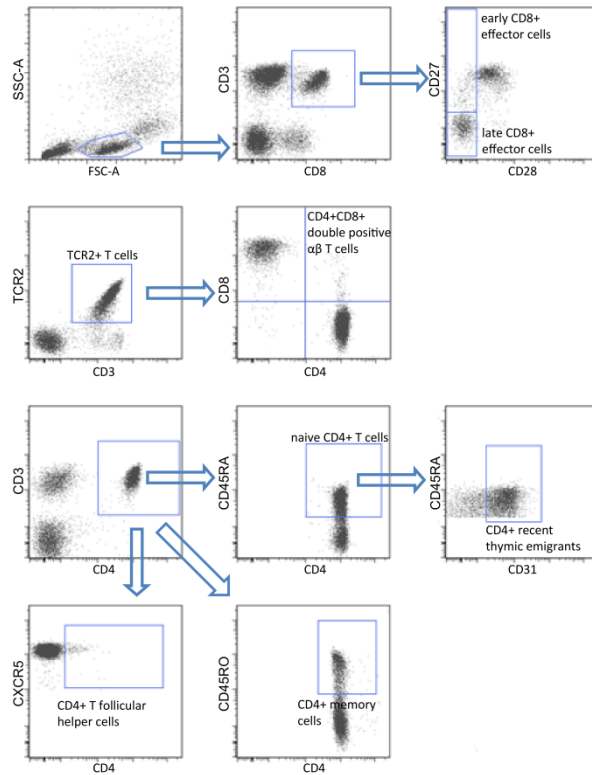


Figure S2. Strategy to measure T cell subsets. After doublet exclusion (not shown) and gating on lymphocytes based on SSC and FSC, we selected CD3⁺ T cells with subsequent gating based on TCR2, CD4, CD8, CD27, CD28, CXCR5, CD45RA and CD45RO expression.

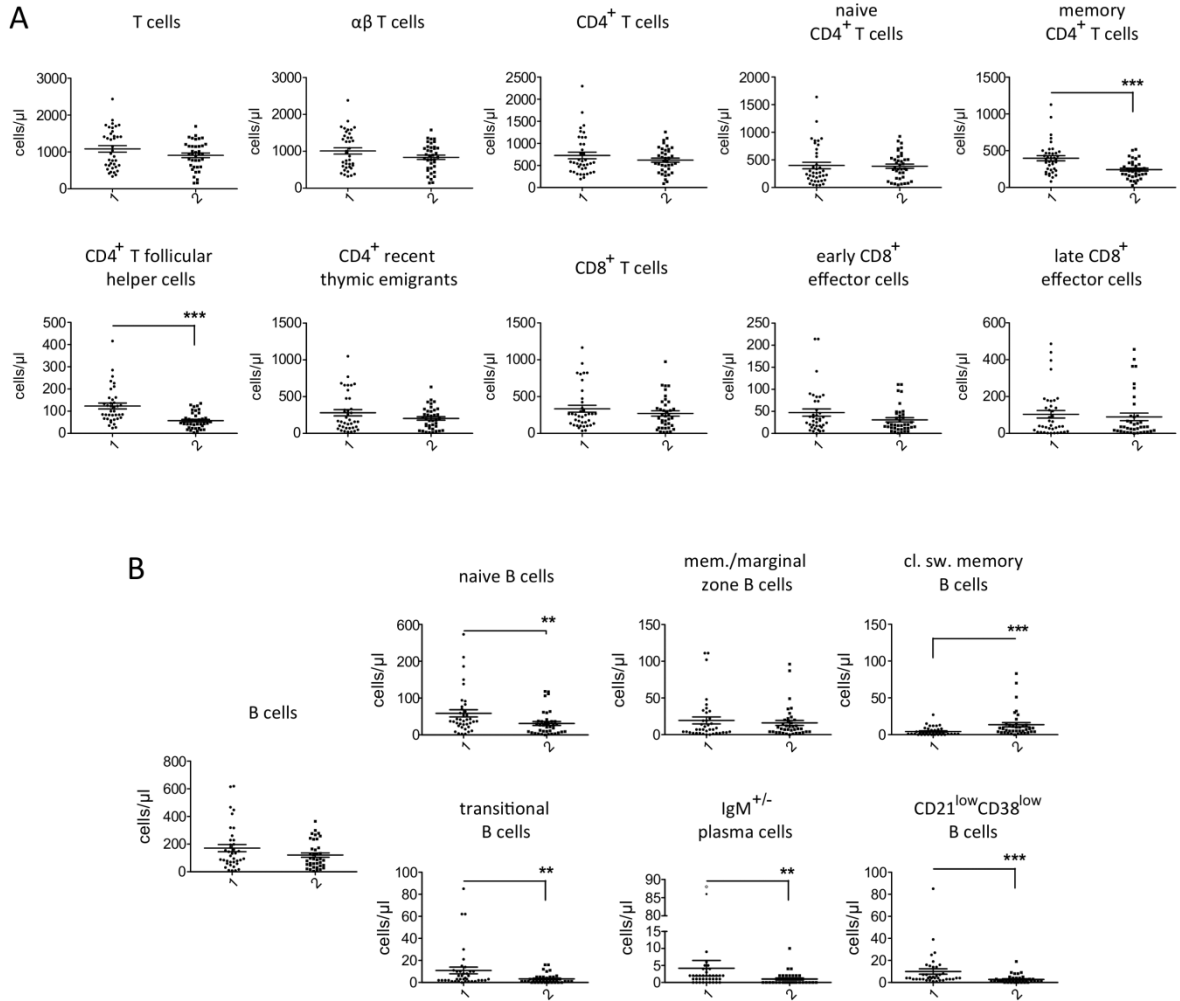


Figure S3. Comparison of absolute T cell (A) and B cell subset counts (B) in patients with primary (1, n=38) and secondary hypogammaglobulinemia (2, n=38 for T cell subsets and n=37 for B cell subsets, as a patient's (pat.nr. 37) B cells could not be differentiated due to their low number; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

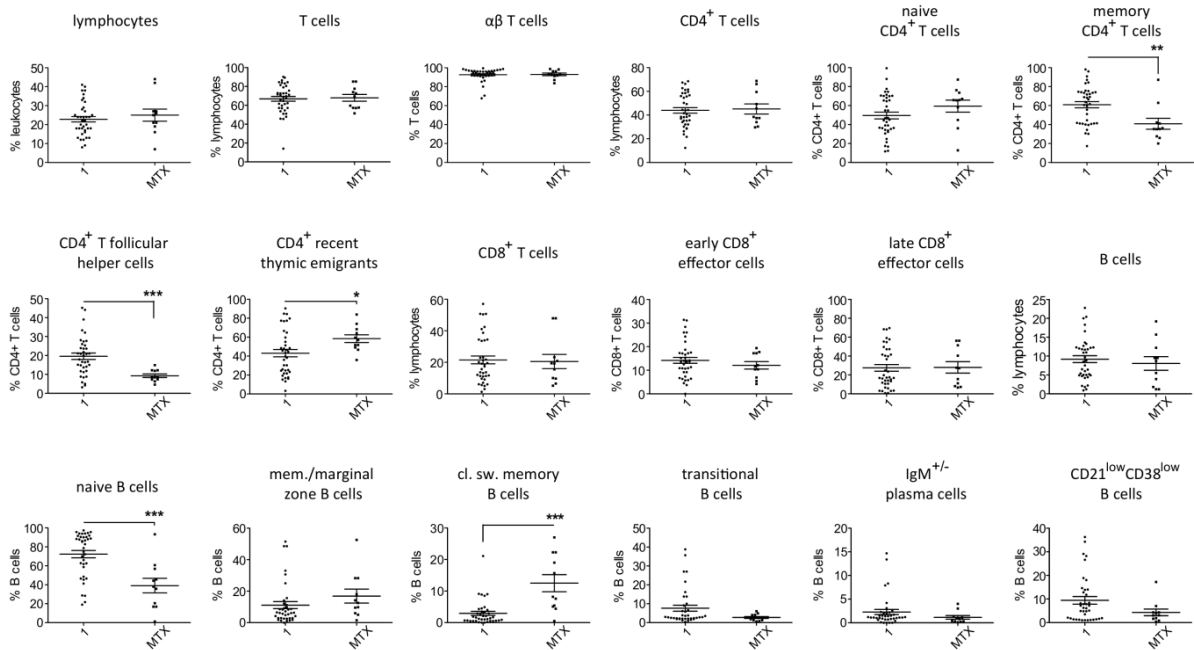


Figure S4. Comparison of B and T cell subsets in patients with primary (1, n=38) and secondary hypogammaglobulinemia due to methotrexate (MTX, n=11; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

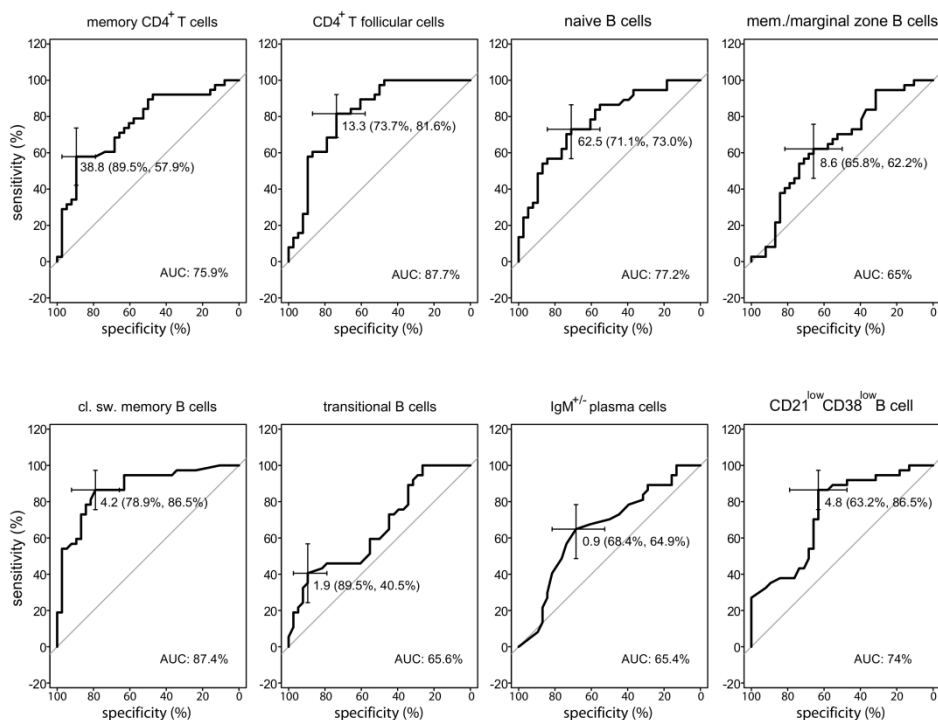


Figure S5. Receiver operating characteristic (ROC) curves and the corresponding area under the curve (AUC) values for the indicated B or T subset counts.

Table S1. Association of lymphocyte subset percentage counts with immunoglobulin values in patients with primary (1) and secondary (2) hypogammaglobulinemia.

memory CD4 ⁺ T cells (% CD4 ⁺ T cells)		
	1	2
IgG	-0.295 (-56.85 - 3.7) / ns	-0.027 (-35.23 - 30.43) / ns
IgA	-0.033 (-35.8 - 29.83) / ns	0.165 (-17.27 - 46.83) / ns
IgM	-0.043 (-36.62 - 28.97) / ns	0.105 (-23.16 - 41.9) / ns
CD4 ⁺ T follicular cells (% CD4 ⁺ T cells)		
	1	2
IgG	-0.172 (-47.35-16.63) / ns	-0.175 (-47.64 - 16.26) / ns
IgA	-0.043 (-36.68 - 28.92) / ns	-0.054 (-37.58 - 27.95) / ns
IgM	-0.051 (-37.36 - 28.2) / ns	0.134 (-20.37 - 44.28) / ns
mem./marginal zone B cells		
	1	2
IgG	0.375 (5.28 - 62.62) / *	-0.03 (-35.97 - 30.56) / ns
IgA	0.248 (-8.79 - 53.29) / ns	0.252 (-8.8 - 54) / ns

IgM	0.464 (15.96 - 68.75) / **	0.123 (-21.89 - 43.8) / ns
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naïve B cells (% B cells)

	1	2
IgG	-0.286 (-56.19 - 0.047) / ns	-0.312 (-58.4 - 2.24) / ns
IgA	-0.262 (-54.36 - 7.3) / ns	-0.513 (-72.22 - -21.65) / **
IgM	-0.409 (-65.02 - -9.3) / *	-0.026 (-35.57 - 30.99) / ns

cl. sw. memory B cells (% B cells)

	1	2
IgG	0.324 (-0.54 - 58.95) / *	0.395 (7.13 - 64.33) / *
IgA	0.325 (-0.4 - 59.05) / *	0.489 (18.61 - 70.67) / **
IgM	0.263 (-7.22 - 54.41) / ns	0.074 (-26.59 - 39.68) / ns

transitional B cells (% B cells)

	1	2
IgG	0.119 (-21.82 - 43.06) / ns	0.156 (-18.68 - 46.47) / ns
IgA	0.101 (-23.5 - 41.6) / ns	0.172 (-17.04 - 47.79) / ns
IgM	-0.018 (-34.49 - 31.19) / ns	-0.171 (-47.72 - 17.13) / ns

IgM⁺ plasma cells (% B cells)

	1	2
IgG	-0.079 (-39.7 - 25.64) / ns	0.122 (-22 - 43.71) / ns
IgA	0.073 (-26.18 - 39.21) / ns	0.006 (-32.72 - 33.87) / ns
IgM	0.088 (-24.76 - 40.49) / ns	0.023 (31.15 - 35.4) / ns

CD21^{low}CD38^{low} B cells (% B cells)

	1	2
IgG	-0.278 (-55.57 - 0.57) / ns	0.09 (-25.09 - 41.03) / ns
IgA	-0.06 (-38.06 - 27.43) / ns	0.092 (-24.89 - 41.2) / ns
IgM	0.062 (-27.2 - 38.28) / ns	-0.008 (-34.01 - 32.58) / ns

(Spearman' s r(95% c.i.)/p-value)

Table S2. lymphocyte subset counts of patients with PID on anti-inflammatory regiments.

Pat. Nr.	Memory CD4 ⁺ T cells (% CD4 ⁺ T cells)	CD4 ⁺ T follicular helper cells (% CD4 ⁺ T cells)	Naïve B cells (% B cells)	Mem./ marginal zone B cells (% B cells)	cl. sw. memory B cells (% B cells)	Transitional B cells (% B cells)	IgM ⁺ plasma cells (% B cells)	CD21 ^{low} B cells (% B cells)	Indication	Therapy
1	83.5	21.1	94	1.3	0.5	13.8	0.4	18	GLILD	AZA
7	74.1	23.6	90.4	8.3	0.5	2.2	1.1	4.8	PsA	MTX
14	40.6	15.1	97.4	1.8	0	35.7	1.4	1.4	GLILD	Prednisolone (5 mg)
20	75.4	13.7	85.4	4.2	2.1	2.1	0.1	6.2	IBD (Crohn's)	MSZ + prednisolone

									like)	(5 mg)
21	71.7	25.3	19	1.1	0.1	1.3	1.4	13.9	GLILD	AZA
25	34.4	33.3	91.5	4.2	2.1	2.1	4.2	2.1	perSpA	MTX

AZA, azathioprine; GLILD, *Granulomatous-lymphocytic interstitial lung disease*; IBD, inflammatory bowel disease; MSZ, mesalazine; MTX, methotrexate; pat. nr., patient number; perPsA, periphere spondyloarthritis; PsA, psoriasis arthritis