Relations between T- and B-lymphocyte abnormalities in CVID patients and their age dependency

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Common variable immunodeficiency (CVID)

- Clinically heterogeneous group of primary humoral immunodeficiency diseases, characterized by impaired antibody production ⇒ decreased levels of IgG, IgA and sometimes also IgM.
- B-cell percentages range from very low to normal
- Etiology unknown (except for deficits ICOS, BAFF-R, TACI).
- Various T- and B-lymphocyte abnormalities were described: defective T-cell activation and impaired cytokine production, abnormal T-cell phenotypes (for example: reduction number of CD45RA, CD62L, increasing number of CD45RO and HLA-DR). For B-cell: reduced number of memory cell – CD27+IgD-IgM-.

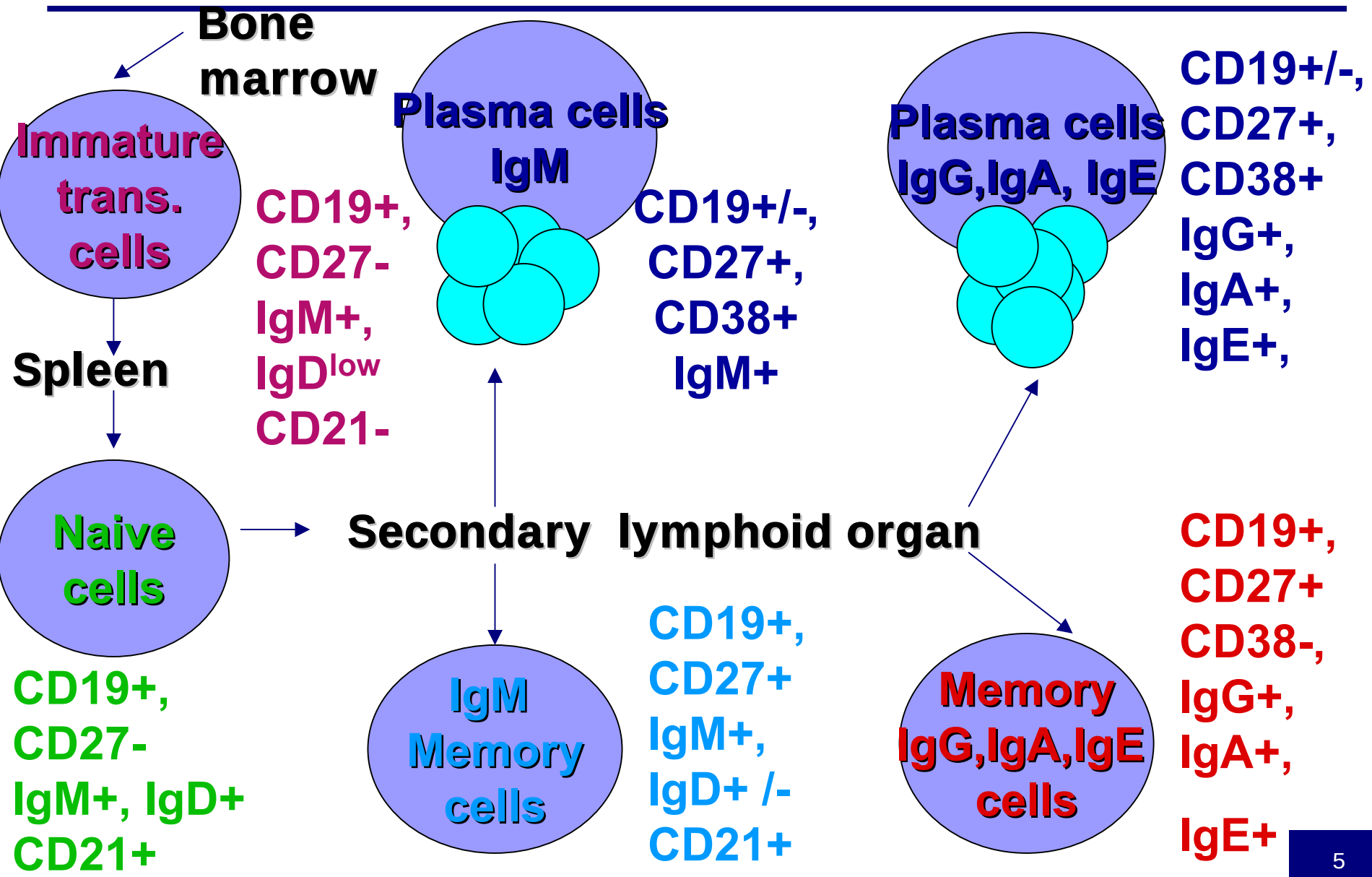
Goals

- A division of CVID patients according to „Freiburg classification“ - based on flow cytometric analysis markers associated with B cell development (CD21, CD27, IgD, IgM).
- Analyse CD4+ T lymphocyte subpopulations, which characterize T-cell development (CD45RA, CD45RO, CD62L) and activation (CD25, CD27, CD28, CD29, HLA-DR) for individual subgroup of CVID patients and controls.
- Analysis of mutual relations between numbers of T and B cells in various developmental and activation stages.
- Based on diminution of memory B cells new classification of CVID patients into more homogeneus patient subgroups was created in Freiburg.

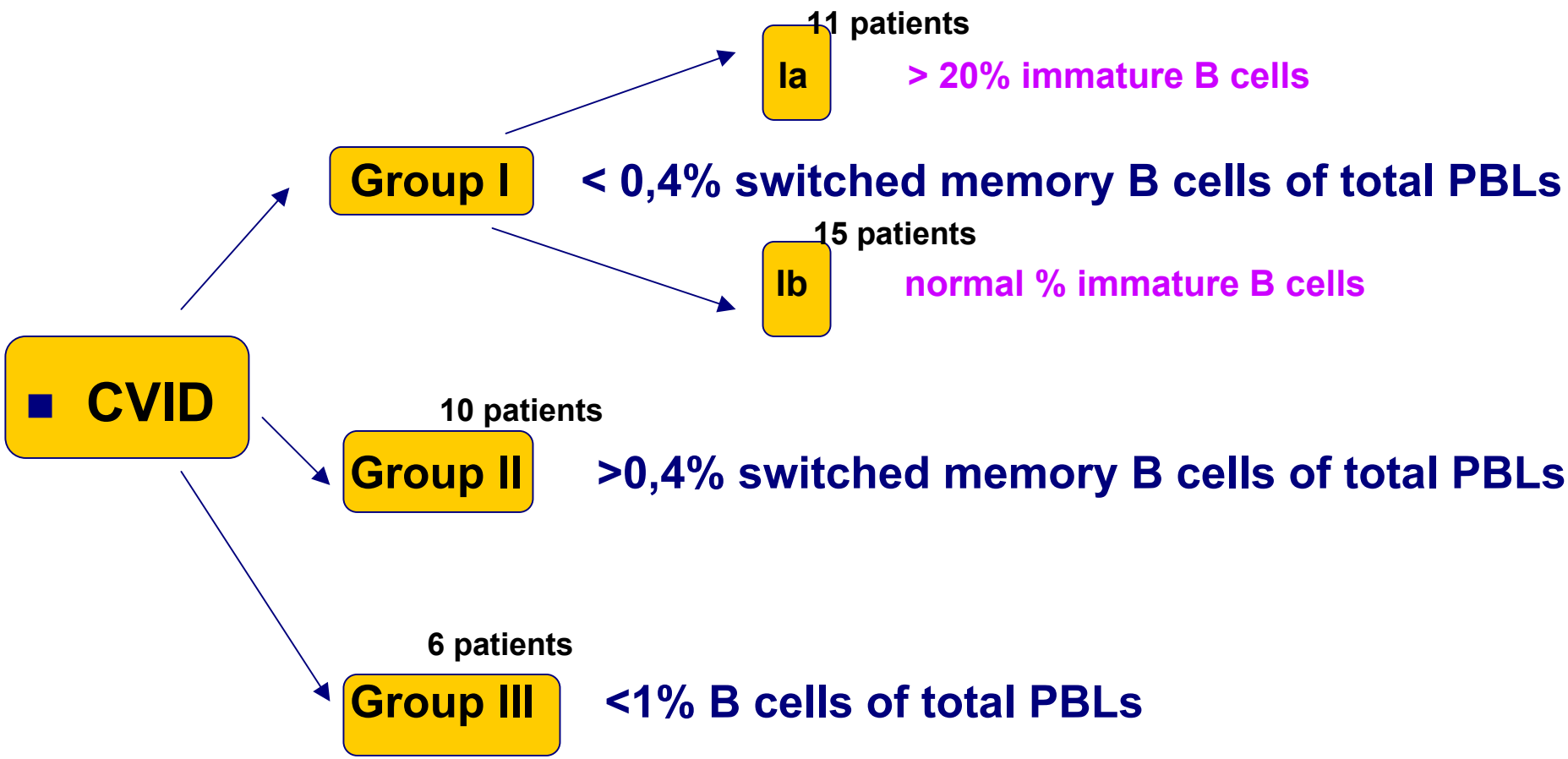
Patients and methods

- 42 CVID patients (10-75 years, mean 43,7±15 years; 28 females, 13 males)
- 33 healthy controls (21-59 years, mean 37,5 ±10,7 years; 26 females, 7 males)
- For B cell analysis - isolation of peripheral blood mononuclear cells (density gradient centrifugation) and stained by standard procedure by flow cytometry
- For T cell analysis - whole blood stained by standard procedure by flow cytometry
- Antibodies: anti: CD25, 27, 28, 29, 62L FITC,
  anti: CD45RA, CD45RO, CD21, HLA-DR, IgD PE
  anti: CD3, IgM PC5 and anti: CD4, 8, 19 ECD
- Phenotyping by four-color cytometer Coulter EPICS MCL

B-cell differentiation



„Freiburg“ classification



CD4+ T-lymphocyte subpopulation

	Ia (Mean ± SD)	Ib (Mean ± SD)	II (Mean ± SD)	III (Mean ± SD)	Control (Mean ± SD)
CD4+	40.3 ± 11.0	§ 36.8 ± 10.0	§ 34.2 ± 6.5	§ 36.4 ± 8.9	47.2 ± 7.7
% CD27+ of CD4+	§ 68.1 ± 15.2	88.0 ± 7.2	84.5 ± 9.0	§ 80.0 ± 9.7	89.6 ± 6.3
% CD28+ of CD4+	92.7 ± 5.9	93.6 ± 4.4	93.8 ± 8.1	92.1 ± 8.3	96.7 ± 4.7
% CD29+ of CD4+	§ 72.0 ± 21.7	58.4 ± 15.4	63.6 ± 10.0	58.0 ± 17.7	49.9 ± 11.0
% CD62L+ of CD4+	§ 45.8 ± 18.6	73.7 ± 14.6	69.5 ± 12.6	72.2 ± 17.5	74.2 ± 10.9
% CD45RA+ of CD4+	§ 8.1 ± 6.9	37.3 ± 15.7	§ 24.7 ± 10.8	§ 21.1 ± 17.2	46.2 ± 13.9
% CD45RO+ of CD4+	§ 92.5 ± 7.6	§ 67.9 ± 16.7	§ 75.1 ± 12.3	§ 76.5 ± 18.5	55.7 ± 14.3
% CD25+ of CD4+	3.9 ± 2.7	§ 4.4 ± 2.6	§ 3.8 ± 1.8	2.0 ± 1.0	1.9 ± 0.8
% HLA DR+ of CD4+	§ 23.3 ± 10.5	10.7 ± 5.9	§ 12.4 ± 6.3	§ 11.1 ± 6.0	5.3 ± 3.0

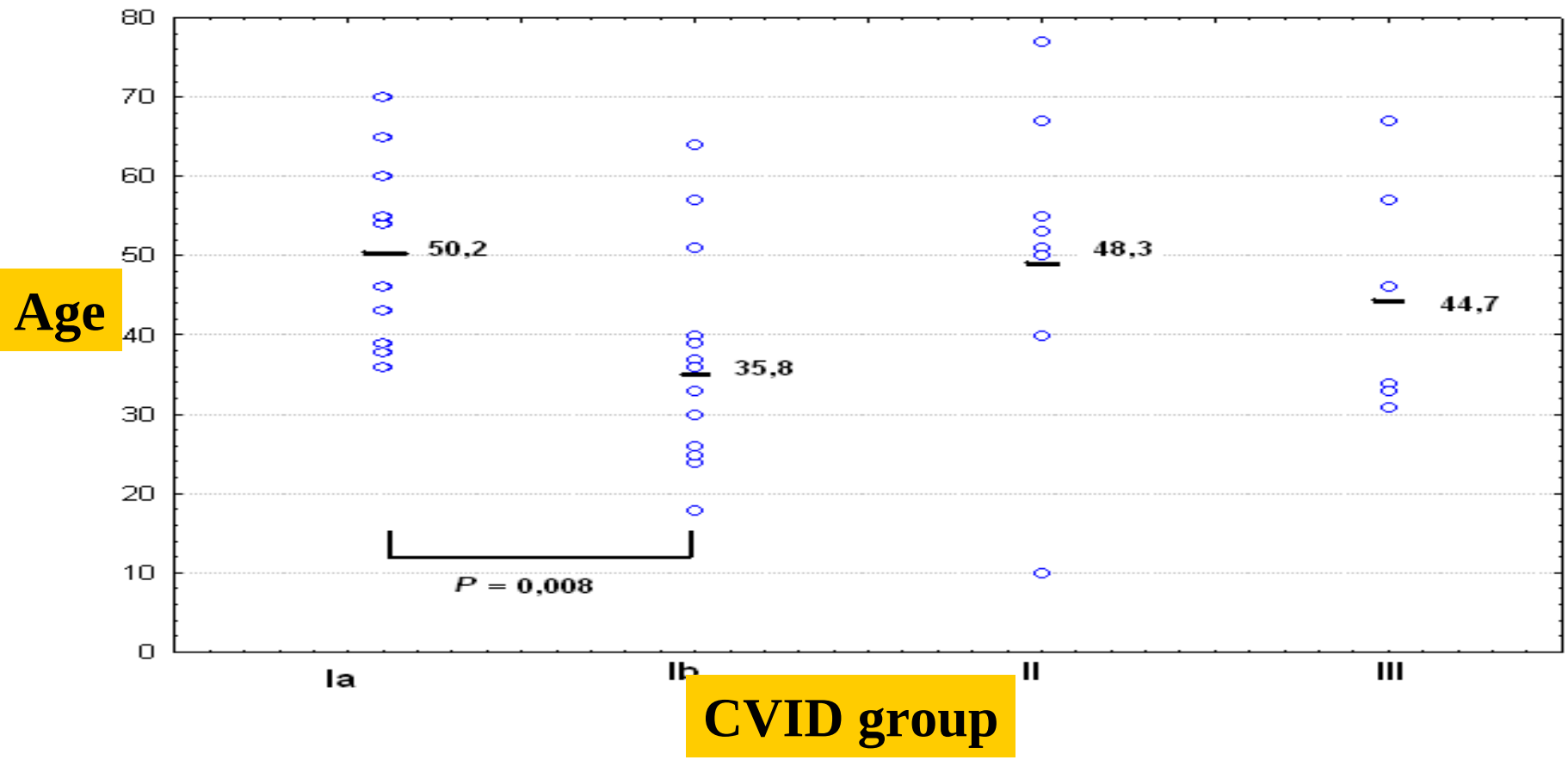
Correlations between the expresion of maturation/activation markers on B and T lymphocytes

Immature B cells

Memory B cells

	IgM+21- of CD19		IgD-27+ of CD19	
	R	*P*	R	*P*
CD4	-0.063582	0.712574	0.091912	0.610953
%CD27 of CD4+	-0.690907	**0.000003**	-0.213235	0.233467
%CD28 of CD4+	-0.313791	0.062366	-0.065508	0.717207
%CD29 of CD4+	0.498359	**0.001979**	0.167447	0.351638
%CD62L of CD4+	-0.735440	**0.000000**	-0.107955	0.549846
%CD45RA of CD4+	-0.643027	**0.000023**	-0.187578	0.295874
%CD45RO of CD4+	0.628869	**0.000040**	0.191025	0.286926
%CD25 of CD4+	-0.130768	0.447141	-0.180329	0.315266
% HLA-DR of CD4+	0.545981	**0.000572**	0.009358	0.958778

Age distribution in CVID subgroups

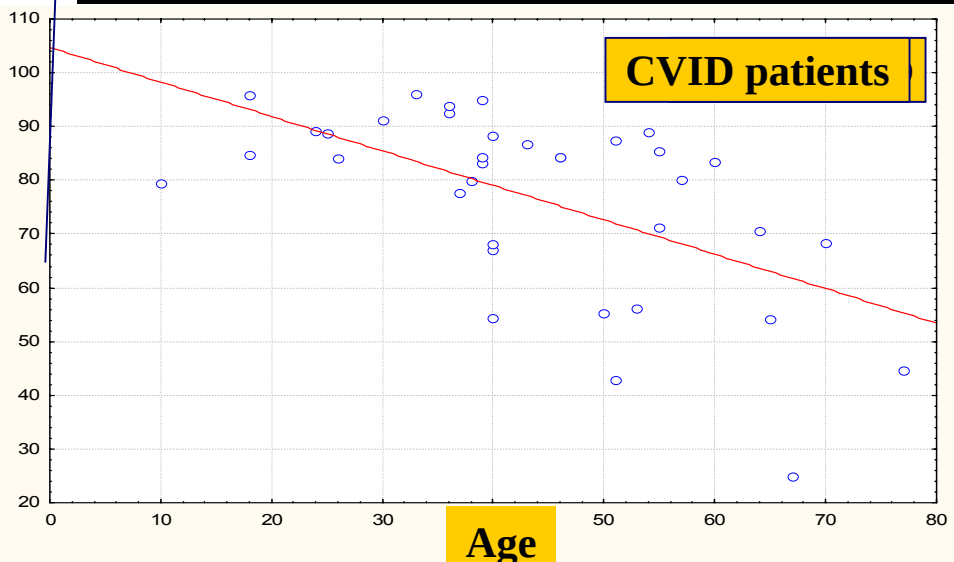


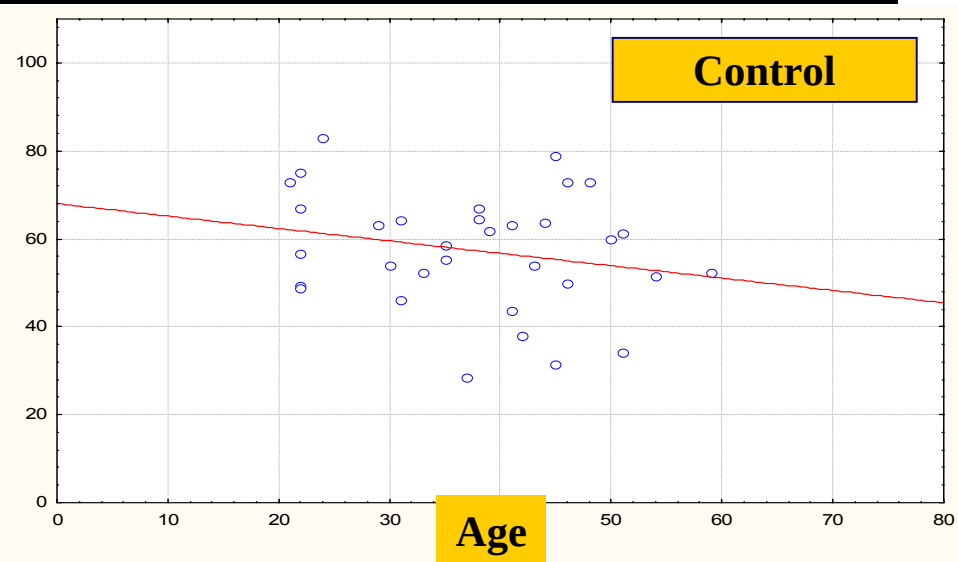
Age dependent correlations in the CVID groups and control groups

Naive B cell

IgM memory B cells

	Age CVID		Age Controls	
	R	p	R	p
IgD+CD27-of CD19	**-0.580504**	**0.000206**	-0.193954	0.279461
IgD+CD27+of CD19	**0.571227**	**0.000274**	0.098819	0.584291
%CD27+ of CD4+	**-0.524295**	**0.000365**	0.064986	0.719368
%CD45RA+ of CD4+	**-0.570612**	**0.000080**	-0.416304	0.015961
%CD45RO + of CD4+	**0.494119**	**0.000882**	**0.656032**	**0.000034**





Conclusion

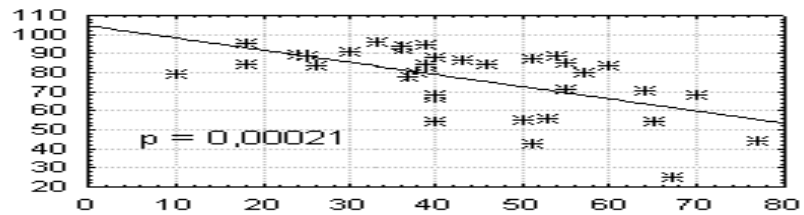
- T- and B-cell abnormalities in CVID patients are partially related to each other (correlation between immature B cells and CD4+ T activation (CD27, CD29, HLA-DR) and differentiation (CD45RA, CD45RO, CD62L))
- Abnormalities in B-cell subpopulation in CVID patients are not definitive but may evolve with the age of patients
- B-cell based classification of CVID patients may be age-related

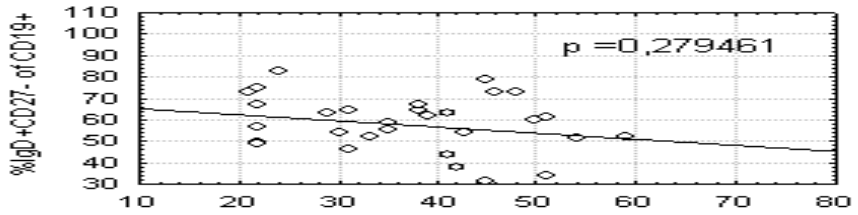
Thank you for your attention

Age dependent correlations in the CVID groups and control groups

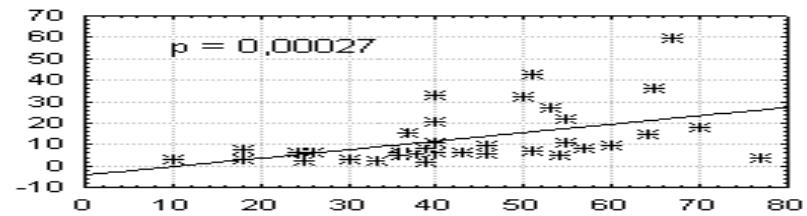
Patients CVID | **Controls**

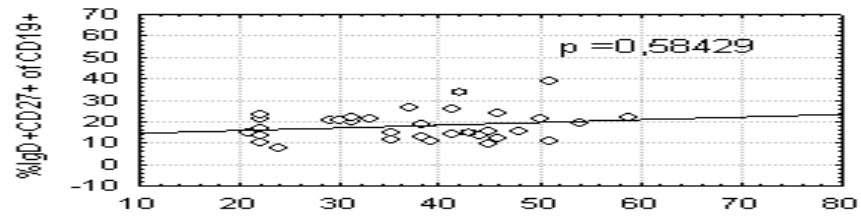
% Naive B-cells



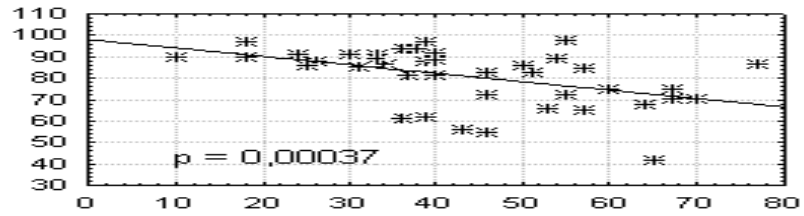


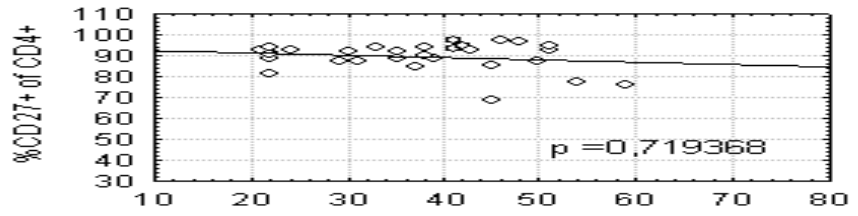
% IgM memory B-cells



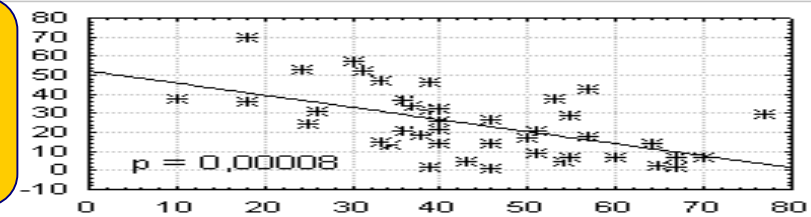


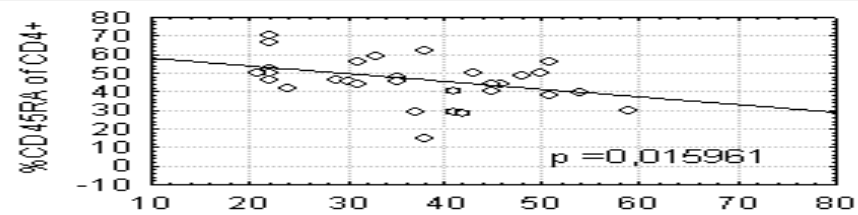
% CD27+ of CD4+



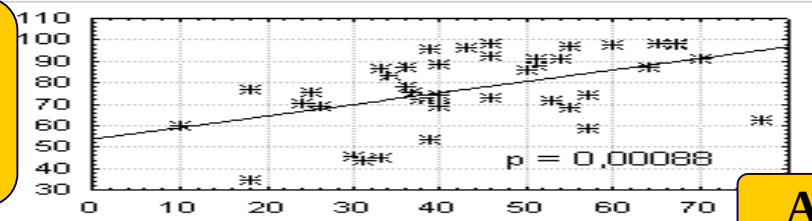


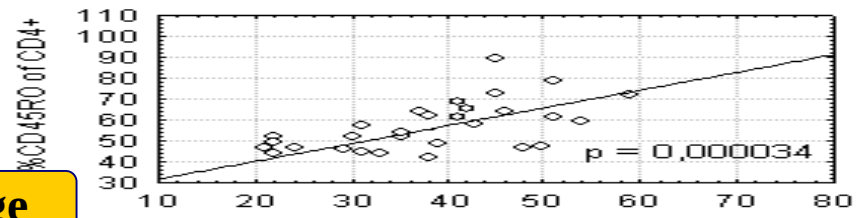
% CD45RA+ of CD4+





% CD45RO+ of CD4+





Age

CD8+ T-lymphocyte subpopulation

	Ia (Mean ± SD)	Ib (Mean ± SD)	II (Mean ± SD)	III (Mean ± SD)	Control (Mean ± SD)
CD8+	§ 36.8 ± 9.0	§ 32.7 ± 10.2	§ 25.9 ± 8.8	§ 43.9 ± 11.4	18.3 ± 5.1
% CD27+ of CD8+	§ 31.3 ± 16.0	§ 52.6 ± 22.1	53.4 ± 19.8	§ 29.2 ± 16.7	68.9 ± 18.0
% CD28+ of CD8+	§ 24.4 ± 11.8	§ 43.9 ± 21.7	§ 47.6 ± 19.0	§ 22.7 ± 12.8	68.0 ± 19.4
% CD29+ of CD8+	90.8 ± 9.9	§ 65.9 ± 26.7	85.0 ± 19.6	85.5 ± 21.7	95.5 ± 4.3
% CD62L+ of CD8+	30.5 ± 15.4	41.1 ± 23.1	40.8 ± 15.5	24.4 ± 15.1	42.7 ± 16.7
% CD45RA+ of CD8+	47.5 ± 14.5	61.2 ± 12.3	48.2 ± 13.5	50.6 ± 20.5	59.0 ± 14.1
% CD45RO+ of CD8+	49.4 ± 12.0	36.7 ± 11.9	48.2 ± 13.9	46.5 ± 19.9	39.8 ± 13.3
%HLA DR+ of CD8+	§ 26.6 ± 7.9	19.0 ± 21.9	§ 18.8 ± 10.5	§ 23.6 ± 12.5	7.1 ± 6.9
%CD38+ of CD8+	§ 34.1 ± 14.6	§ 30.6 ± 19.3	§ 23.7 ± 13.7	§ 31.8 ± 13.1	11.3 ± 6.1
%CD57+ of CD8+	§ 41.2 ± 8.2	§ 28.5 ± 16.8	§ 29.8 ± 12.7	§ 49.1 ± 10.2	16.2 ± 12.9

Correlations between the expresion of maturation/activation markers on B and CD8+T lymphocytes

	IgM+21low of CD19		IgD-27+ of CD19	
	R	*P*	R	*P*
CD8	0.220592	0.196068	-0.047794	0.791687
%CD27 of CD8+	**-0.486744**	**0.002610**	0.109291	0.544886
%CD28 of CD8+	-0.404118	0.014509	-0.080214	0.657229
%CD29 of CD8+	0.336165	0.045009	0.008356	0.963191
%CD62L of CD8+	-0.292149	0.083813	-0.115976	0.520408
%CD45RA of CD8+	-0.245560	0.148860	-0.266377	0.134019
%CD45RO of CD8+	0.216988	0.203660	0.202540	0.258305
% HLA-DR of CD8+	**0.524067**	**0.001036**	0.027409	0.879654
%CD38 of CD8+	0.185586	0.278514	-0.075535	0.676107
%CD57 of CD8+	**0.589356**	**0.000156**	-0.017380	0.923524

Acknowledgments

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I am most grateful tu my tutor prof. Jiří Litzman for advice and comments. I thank my co-workers: Mária Šárfyová, Luděk Bláha and Vojtěch Thon and my family for a patience.

Statistical analysis

Statistical analysis

The differences between the groups were evaluated by Analysis of Variance (ANOVA) followed by Dunnet's (comparison between patients and control group) or Newman-Keul's (general comparison between groups of patients) contrasts. The results were confirmed using non-parametric Mann-Whitney test. *P*-values less than 0.05 were considered statistically significant in all statistical analyses. Student's t-test and Fisher's exact test were used when appropriate.

Correlations between variables were evaluated using Spearman's correlation coefficient (R_s) and the results were revised by Bonferroni's correction for each group of lymphocytes – CD4+, CD8+, CD19+ [19]. Statistical package STATISTICA (StatSoft, Inc.) version 7 was used.

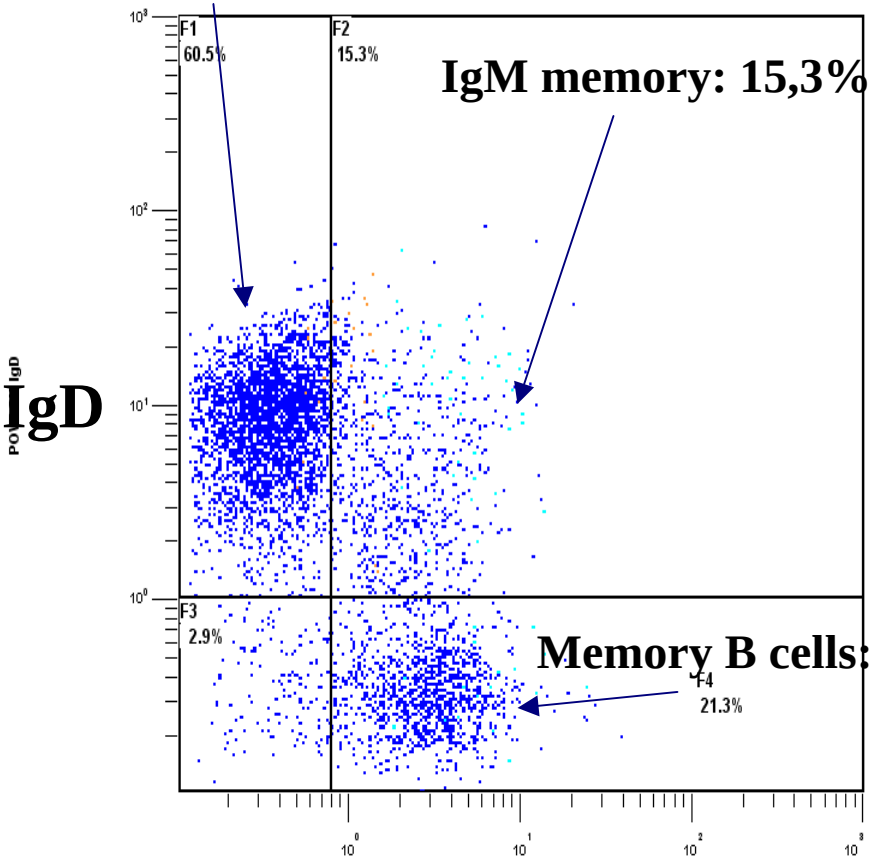
Various stages of B cell differentiation

Healthy control

Naive B cells: 60,5%

IgM memory: 15,3%

Memory B cells: 21,3%

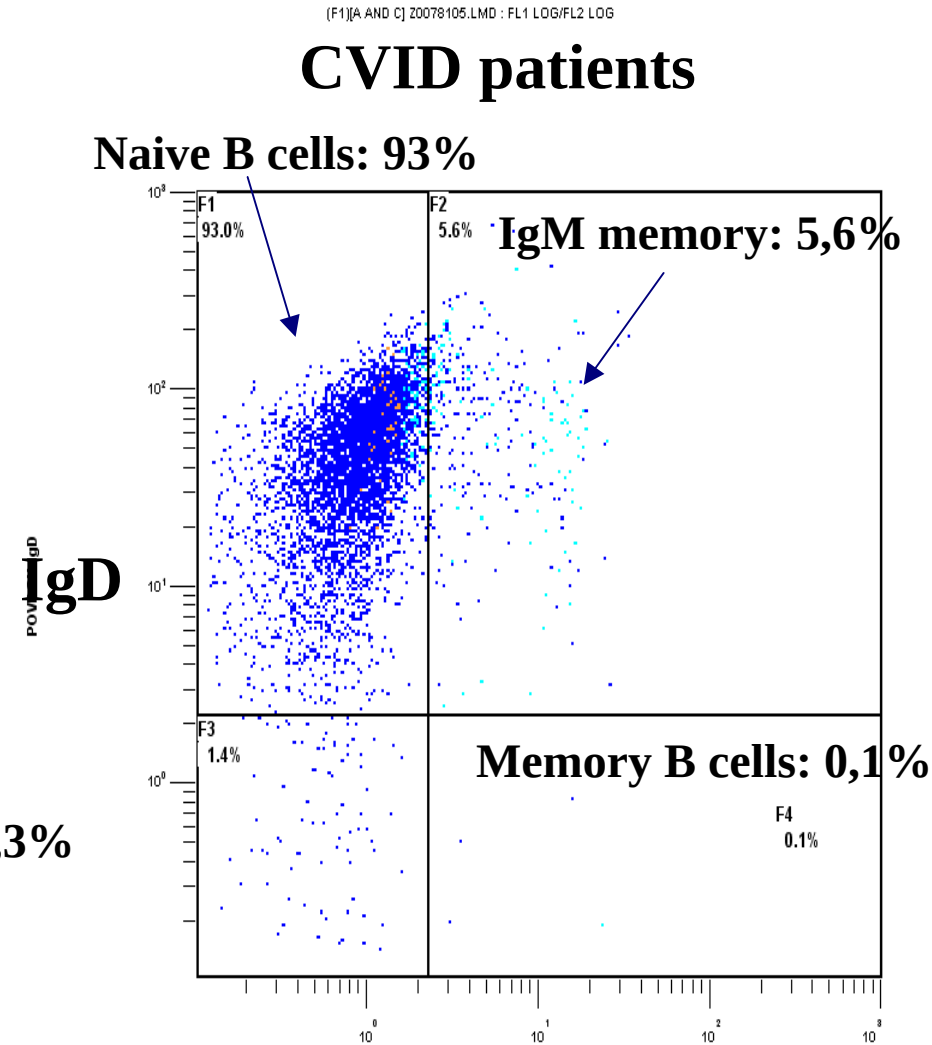


CVID patients

Naive B cells: 93%

IgM memory: 5,6%

Memory B cells: 0,1%



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Immature B cells

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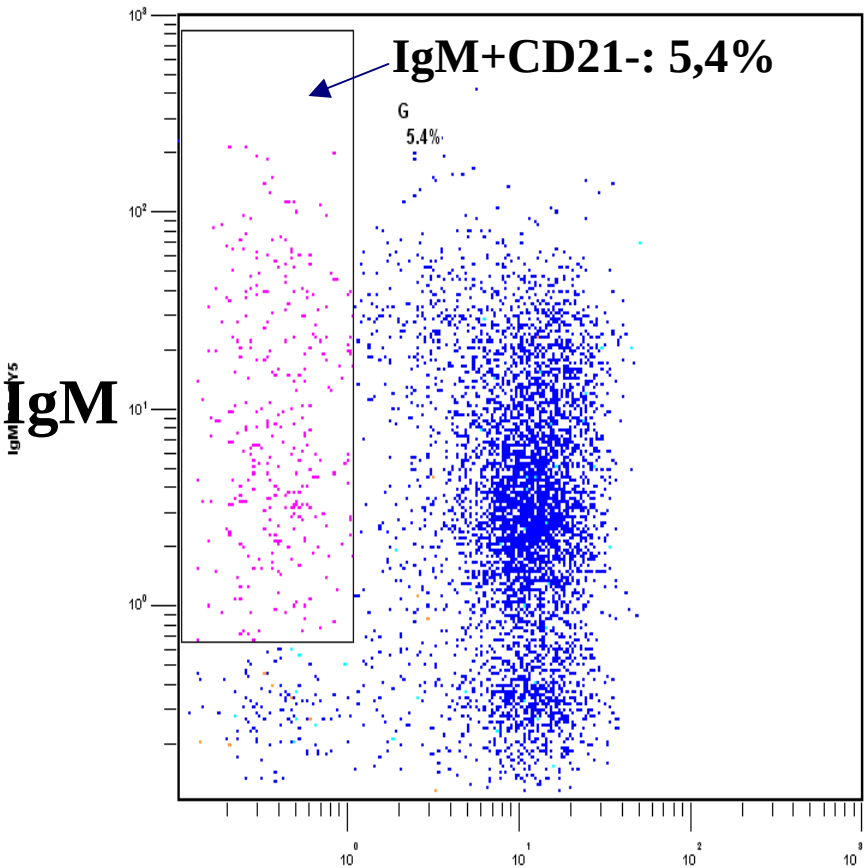
Healthy control



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CVID patient