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Regulatory B cells in CVID patients fail to suppress multifunctional IFN-γ$^+$TNF-α$^+$CD4$^+$ T cells differentiation

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ABSTRACT

Common variable immunodeficiency (CVID) refers to primary hypogammaglobulinemia with unknown pathogenesis. Although there is evidence for intrinsic B cell defects in some CVID patient groups, various abnormalities in cytokine production by T cells in CVID patients are frequently observed. Here, we demonstrate a relationship in the production of pro-inflammatory Th1 cytokines and regulatory B cells producing IL-10 between CVID patients and healthy controls. We describe CD19$^+$CD24^{hi}CD38^{hi}IL-10$^+$ regulatory B cells generated after T cell stimulation of human peripheral blood lymphocytes ex vivo are able to suppress IFN-γ$^+$TNF-α$^+$ producing CD4$^+$ T cells. This process is impaired in CVID patients, who present with both low numbers of CD19$^+$CD24^{hi}CD38^{hi}IL-10$^+$ B cells and increased numbers of IFN-γ$^+$TNF-α$^+$CD4$^+$ T cells. Disruption of the regulatory B cell response to T cell stimulation explains the excessive T cell activation regarded as an immunoregulatory abnormality that is a frequent finding in CVID patients.

1. Introduction

Common variable immunodeficiency (CVID) encompasses a clinically heterogeneous group of primary antibody immunodeficiency diseases [1]. It is characterized by decreased serum levels of IgG, IgA and/or IgM and impaired antibody responses to antigen stimulation and/or vaccination [2]. Clinically it is accompanied by recurrent or chronic bacterial infections, inflammatory and autoimmune disorders and increased risk of malignancy [1].

Although various abnormalities in the number and function of lymphocytes and dendritic cells [3,4] have been described, the immunopathological mechanism leading to disturbed antibody production in CVID patients has not yet been elucidated fully. Several studies have shown alterations and defects in T cells: reduced proportion of naïve CD4$^+$ CD45RA$^+$ T cells, increased number of CD4$^+$CD45RO$^+$ memory T cells and increased number of CD4$^+$ HLA-DR$^+$ activated T cells, defects in TCR repertoire, and changes in regulatory T cells [5–10]. The change in CD4$^+$ T lymphocyte markers indicates a shift to activated and memory phenotypes. The increase in activation of CD4$^+$ T cells is also reflected by the increase in production of interferon (IFN)-γ by CD4$^+$ T cells in CVID patients upon CD3 or PMA/PMH stimulation after 24 h and upon phorbol myristate acetate (PMA)/Ionomycine stimulation after 12 h [11,12]. Increased levels of (tumor necrosing factor) TNF-α and also IFN-γ after stimulation of T-cells were reported in several studies in CVID patients [13]. IFN-γ and TNF-α are both considered crucial Th1 cytokines (10). By contrast, B cells in CVID patients have increased number of naïve follicular B cells but low numbers of memory B cells and plasmablasts [14]. A subset of CVID patients presents with markedly increased transitional B cells [15]. A significant portion of the B lymphocytes in CVID patients are anergic cells [16]. The alterations found in the B-cell compartment are unexpectedly stably detected over 4–11 months in CVID patients [15].

Apart from antibody production and antigen presentation, B lymphocytes also secrete cytokines. Similarly to T regulatory cells, a distinct subpopulation of B lymphocytes, now called regulatory B cells (B reg), can produce anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β and can express inhibitory molecules that suppress T cells and autoreactive B cells in a cell-to-cell contact-dependent manner [17]. The first observations of B cell regulatory function in experimental autoimmune encephalomyelitis in mice were carried out in 1996 by Janeway and colleagues [18]. The term "regulatory B cells" was first "popularized" by Mizoguchi and Bhan, or Ono and Hamaoka in 2000 for a mouse B cell subset that secretes IL-10 and suppresses progression of inflammation [19,20]. In recent years, regulatory B cells have been intensively studied in various models of inflammation, cancer, transplantation and in human autoimmune diseases [21–24]. Various populations of B cells producing IL-10 have been

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detected among transitional 2-marginal zone precursor cells (T2-MZP), follicular B cells (FO), marginal zone B cells (MZ) and plasma cells [24]. In 2010, Mauri and colleagues demonstrated that the $CD19^+$ $CD24^{hi}CD38^{hi}$ subset of human B cells comprises the highest fraction of IL-10-producing B cells upon CD40 stimulation in human peripheral blood from healthy individuals [17]. $CD19^+CD24^{hi}CD38^{hi}$ B cells have a phenotype that was previously assigned to immature transitional B cells [25]. These B cells were shown to suppress the production of pro-inflammatory cytokines (TNF-α and IFN-γ in $CD4^+$ T cells) [17].

Although an increase in Th1 cytokine production in CVID patients was described (10), regulatory B cells in CVID patients have not yet been studied. Here we focused on a detailed analysis of the interaction between regulatory B cells and $CD4^+$ T cells in peripheral blood in CVID patients and compared it to healthy donors.

2. Material and methods

2.1. Patients and controls

PBMCs from 26 CVID patients were examined. All patients were Caucasians of Czech origin and fulfilled the PAGID (Pan-American Group for Immunodeficiency)/ESID (European Society for Immunodeficiencies) diagnostic criteria for CVID [2]. Basic characteristics of the patients are given on Table 1. In one patient the p.C104R mutation of the *TNFRSF13B* gene (coding for TACI) was observed; no mutations in the genes *ICOS* (performed in 14 patients) or *TNFRSF13C* (performed in 2 patients) were recorded.

The patients were on regular intravenous (IVIG, given once in 3–4 weeks) or subcutaneous (given 1–2 times per week) immunoglobulin replacement treatment (see Table 1).

In case of patients on IVIG, blood samples were collected before the IVIG infusion was given. Healthy donors were studied in parallel with the patients.

All samples were collected during apparent acute infection-free period defined as worsening cough, rhinitis, or presence of new symptoms suspicious of respiratory, gastrointestinal or urinary tract infections or significant increase in CRP above the levels typically observed in the given patient. One patient was treated by methylprednisolone 4 mg/day, and leflunomide 20 mg/day because of arthritis and demyelinating disease. No patient was under cytostatic treatment at the time of the study.

2.2. Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from EDTA blood by density gradient (Lymphoprep; Nycomed Pharma AS, Oslo, Norway) and washed twice with phosphate-buffered saline. Isolated PBMCs were re-suspended in fetal calf serum with 5% dimethyl sulfoxide at the concentration of 1×10^7/ml before cryopreservation in liquid nitrogen.

2.3. Cell culture

2.3.1. Stimulation with plate-bound CD3 mAb

Thawed PBMCs were stimulated with 0.5 μg/ml purified plate-bound CD3 monoclonal antibody — mAb (Hit-3a) (BioLegend, San Diego, USA) for 72 h. This stimulation led to activation and expression of CD154 on $CD4^+$ T-cells. The interaction between CD40 on B cells and CD154 on $CD4^+$ T cells resulted in the production of IL-10 in B regulatory cells [17]. For costimulation and detection of

Table 1
Clinical and laboratory characteristics of evaluated CVID patients.

Patient No.	Sex	Age (years)	EUROclass classification	Absolute B cells count (10^6/ml)	%CD24hiCD38hi/CD19+ ex vivo	%CD24hiCD38hi/CD19+ after stimulation	%IL-10+/CD24hiCD38hi after stimulation	IFN-γ+TNF-α+/CD4+	Immunoglobulin dose (mg/kg/4 weeks)	Route of administration	IgG at diagnosis (g/l)	IgA at diagnosis (g/l)	IgM at diagnosis (g/l)	Splenomegaly*	Autoimmunity	Bronchiectasis	Malignancy
1	M	61	smB-/21low	0.128	4.9	31.7	5.5	23.2	394	iv	0.42	<0.02	0.33	Yes	No	Yes	No
2	M	55	smB-/21low	0.012	12.8	27.1	10.6	21.5	480	iv	1.15	0.08	0.21	Yes	No	Yes	Hodgkin lymphoma**
3	F	80	smB-/21low	0.595	0.3	39.0	8.0	34.8	373	iv	0.59	<0.05	0.07	No	Pernicious anemia	Yes	No
4	F	65	smB-/21low	0.182	13.0	9.8	8.9	12.6	232	iv	0.75	<0.08	0.13	Yes	Thrombocytopenia	Yes	No
5	M	47	smB-/21low	0.039	5.3	30.3	19.4	36.2	385	iv	1.39	<0.01	<0.05	Yes	No	No	No
6	F	50	smB-/21low	0.057	8.4	38.7	12.6	13.3	458	iv	3.36	<0.01	0.32	Yes	No	No	No
7	F	56	smB-/21low	0.143	6.8	21.8	10.4	6.1	600	iv	1.84	<0.08	0.27	Yes	Thrombocytopenia	Yes	No
8	F	33	smB-/21low	0.141	6.9	62.0	15.2	8.5	471	iv	0.08	0.04	<0.05	Yes	No	No	No
9	F	68	smB-/21low	0.054	2.6	32.9	27.8	6.9	381	iv	4.34	0.09	0.08	No	SLE, vitiligo, DM-I	Yes	Basalioma**
10	F	49	smB-/21low	0.14	6.3	39.0	24.6	11.0	472	iv	1.13	<0.08	<0.08	No	No	Yes	No
11	F	50	smB-/21norm	0.202	3.8	21.7	4.2	20.4	424	sc	1.73	<0.08	0.08	Yes	No	No	No
12	M	65	smB-/21norm	0.175	6.4	48.3	13.5	15.9	220	iv	2.33	0.11	0.09	Yes	No	No	No
13	M	27	smB-/21norm	0.253	8.8	50.3	12.6	23.7	323	iv	1.74	<0.01	0.22	Yes	No	No	No
14	F	67	smB-/21norm	0.117	7.5	34.3	15.3	38.8	301	iv	1.99	0.14	<0.04	No	No	No	No
15	M	36	smB-/21norm	0.198	23.6	30.5	27.8	7.9	360	iv	3.19	<0.08	0.38	Yes	No	Yes	No
16	F	36	smB-/21norm	0.372	6.2	51.6	16.7	18.9	360	sc	4.01	<0.01	<0.08	No	No	No	No
17	F	45	smB-/21norm	0.271	7.4	44.7	21.3	14.3	444	iv	1.68	<0.08	0.23	Yes	No	No	No
18	F	26	smB-/21norm	0.039	2.6	30.7	17.4	6.4	481	iv	0.66	<0.01	0.13	No	Hypothyreosis	No	No
19	M	40	smB-/21norm	0.066	16.6	38.3	25.0	7.5	280	iv	2.35	<0.01	0.06	No	No	No	No
20	M	40	smB-/21norm	0.129	3.0	30.7	31.1	11.8	350	sc	1.51	<0.01	0.09	Yes	No	No	No
21	M	51	smB+/21low	0.033	3.5	21.1	5.1	23.8	357	iv	1.72	0.34	0.11	No	Pernicious anemia	No	No
22	M	61	smB+/21low	0.043	4.8	25.3	5.6	33.7	420	iv	0.85	<0.08	<0.08	Yes	No	No	No
23	M	33	smB+/21low	0.198	3.0	7.2	5.4	27.9	409	iv	2.51	0.39	<0.05	No	No	No	No
24	F	75	smB+/21low	0.049	5.1	71.2	6.8	26.5	329	iv	2.17	0.20	0.01	Yes	Pernicious anemia	No	Gastric cancer**
25	F	61	smB+/21low	0.11	3.4	42.3	15.7	22.2	270	iv	4.48	<0.01	0.06	No	No	No	No
26	F	30	smB+/21low	0.141	8.1	13.9	18.7	8.7	320	sc	2.05	0.08	0.23	No	Arthritis, demyelination, hypothyreosis	No	No

*Defined as spleen length <11 cm on sonography.
**In remission in the time of the study.
SLE: systemic lupus erythematosus, DM-I: Diabetes mellitus type 1.