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B cells from common variable immunodeficiency patients fail to differentiate to antibody secreting cells in response to TLR9 ligand (CpG-ODN) or anti-CD40 + IL21

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ABSTRACT

Common variable immunodeficiency (CVID) is a primary immunodeficiency characterised by hypogammaglobulinaemia and antibody deficiency to T dependent and independent antigens. Patients suffer from recurrent respiratory infections and poor response to vaccination. Although the underlying molecular defect is unknown, most CVID patients show impaired late B cell differentiation. We investigated B cell differentiation and immunoglobulin secretion induced by two different stimuli: TLR9 specific ligand (CpG-ODN) and anti-CD40 combined with IL21. The contribution of BCR signalling (anti-IgM stimulation) was also evaluated. B cells from CVID patients produced low levels of IgG and IgA in response to both kinds of stimuli that was not restored by anti-IgM. Production of IgM was conserved when cells were stimulated with anti-CD40 and IL21. These results point to a wide signalling defect in B lymphocytes from CVID patients that may be related to their hypogammaglobulinaemia and poor response to vaccination.

1. Introduction

Common variable immunodeficiency (CVID) includes a heterogeneous group of disorders of unknown aetiology characterised by deficient antibody production and recurrent respiratory infections by encapsulated bacteria, mostly *Streptococcus pneumoniae* and *Haemophilus influenzae* and poor response to vaccination. CVID is the most frequent symptomatic primary humoral immunodeficiency and patients benefit from substitutive gammaglobulin therapy [1,2]. The molecular defect underlying this pathology remains obscure, although several genetic mutations and polymorphisms (ICOS, TNFRS13B/TACI, CD19 and Msh5, CD20, CD81) associated with CVID have been described in 10% of the patients [3–5]. As a result of the heterogeneity of the syndrome, a vast array of defects have been described in CVID patients: low numbers and impaired functionality of T cells [6], decreased proliferative response to antigens and mitogens [7], diminished cytokine production [8], altered interactions between T and B cells [9] and even dendritic cell disturbances [10]. However, most CVID patients show abnormal late B cell differentiation to $CD27^+$ memory B cells, switched memory B cells and plasma cells, in spite of having normal numbers of B lymphocytes. Memory B cell deficiencies have been associated with a worse clinical presentation and poor response to vaccines [11,12]. Previous functional in vitro studies have shown that B cells from CVID patients undergo class switch recombination and produce immunoglobulins (Igs) if appropriately stimulated with combinations of anti-CD40, IL10 and/or IL4 [13–15].

The immune response to different pathogens depends on the production of distinct Ig isotypes and several human immunodeficiency diseases are associated with a dysregulated isotype switch. Isotype switching by antigen primed B cells requires two signals: a costimulatory signal from $CD4^+$ T cells (e.g. CD40L) and the contribution of cytokines [16]. Several cytokines like IL4, IL10, IL13, IL21 and TGFβ have been demonstrated as switch factors for the in vitro production of IgG, IgA and IgE by activated B cells [17–19]. Furthermore, following antigenic stimulation, TLR can provide an additional signal for the differentiation of B cells and, for several stimuli even substitute for T cells [20].

IL21 is a type I cytokine that belongs to a family that uses the common cytokine receptor γ-chain as a component of their receptors. IL21 is produced mainly by activated $CD4^+$ T cells [21]. In vitro, IL21 can have positive or negative effects on B cells depending on the presence or absence of other signals. In humans, IL21 was found to be the most potent T cell derived cytokine to induce B cell proliferation and is a potent inductor of plasma cell differentiation if combined with anti-CD40 or anti-CD40 and anti-IgM stimulation [22]. In contrast with IL2 and IL10 that induce plasma cell differentiation of memory B cells stimulated with anti-CD40 [23], IL21 is able to induce plasma cell differentiation and Ig production by naive B cells [22]. IL21 and anti-CD40 induce class switch recombination and secretion of IgG and IgA in pre-switched IgM memory B cells [24,25]. In contrast, stimulation with IL21 and anti-IgM re-

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Table 1
Characteristics of the CVID patients included in the study.

Patient	Age (years)	Sex (male/female)	CD19^{+a} (%)	CD19^{+a} (%)		
				IgD$^+$ CD27$^-$	IgD$^+$ CD27$^+$	IgD-CD27$^+$
1	40	M	5	94	5	0
2	73	F	27	72	27	0, 5
3	59	M	8	96	3	0
4	26	M	15	95	0,5	0, 5
5	26	F	9	87	1,9	1, 7
6	58	F	19	84	5	5
7	64	F	7	73	18	5
8	43	F	16	77	20	1
9	56	F	5	95	1	1
10	40	F	20	86	2	1
11	55	M	2	83	15	0
12	44	F	9	75	17	5
13	26	F	9	84	8	4
14	26	M	18	90	1	2
15	31	F	16	70	2	3
Normal values	–	–	10.7 ± 0.8[b]	61 ± 4.5[b]	10.8 ± 2.8[b]	14.7 ± 2.2[b]

[a] B cells and B cell subpopulations were evaluated as percentage of lymphocytes and percentage of total B cells respectively.
[b] Normal values expressed as mean ± SEM.

sults in B cell death [22]. Recently, Borte et al. [26] demonstrated that a combination of IL21, IL4 and anti-CD40 is able to induce class-switch recombination to IgG and IgA and differentiation of Ig-secreting cells in peripheral blood mononuclear cells (PBMC) from CVID and IgA deficient patients. No mutations in IL21 gene were found. Interestingly, CD138$^+$ defined plasma cells accounted for only a small fraction of overall IgG or IgA production [26]. CD38$^+$ is upregulated during the germinal centre process of B cell stimulation and differentiation induced by T lymphocytes and soluble mediators. Both plasmablasts and plasma cells express CD38 although their expression of CD138 is heterogenous [27].

In this study we evaluated B cell differentiation and Ig production by purified B cells from CVID patients stimulated with two kinds of stimuli: IL21 in combination with anti-CD40 (simulating T cell dependent stimulation) or CpG-ODN (simulating T cell independent stimulation). The contribution of BCR signalling (anti-IgM stimulation) was also evaluated. We found that purified B cells from CVID patients show a defective response to both kinds of stimuli. Irrespectively of their degree of differentiation, secretion of IgG and IgA was diminished in B cells from CVID patients compared to controls, whatever the stimulus used. Production of IgM was conserved when cells were stimulated with anti-CD40 and IL21.

2. Materials and methods

2.1. Patients

Fifteen CVID patients were selected according to diagnostic criteria of the International Union for Immunological Societies scientific group for primary immunodeficiency diseases. Patients received intravenous gammaglobulin therapy every 21 days with the exception of two patients: patient 14 had not received substitutive therapy during the last year and patient 15 had not begun the treatment when the study was started. Patients did not suffer from infections at the time of the study. Peripheral blood samples were collected before gammaglobulin replacement after informed consent. Table 1 summarises patients present age, gender and percentage of total B cells and B cell subpopulations. Sixteen age and sex matched healthy blood donors were included as controls.

2.2. B lymphocyte purification and cell culture

B cells were obtained from PBMC by negative selection using the Dynabeads Untouched^{TM} human B cells separation kit (Dynal, Invitrogen) according to manufacturer instructions. The purity of B cells (median and interquartile range-IQR) was similar in CVID patients and controls (93 IQR: 79–95.5% *vs.* 90 IQR: 85–95%, respectively). Purified B cells were resuspended in RPMI-1640 medium supplemented with 10% heat inactivated foetal calf serum (FCS), glutamine (2 mM), Hepes (200 mM), and antibiotics (penicillin and streptomycin).

Purified B lymphocytes (1×10^5 cells) were added to 96-well plates and stimulated with CpG-ODN2006 (0.6 μg/ml; CpG oligonucleotide type B; InvivoGen), F(ab)2 goat anti-human IgM (5 μg/ml; Jackson ImmunoResearch), recombinant human IL21 (100 ng/ml; Biosource), anti-human CD40/TNFRSF5 antibody (1 μg/ml; R&D Systems) at different combinations.

Cultures were maintained at 37 °C in a 5% CO_2 atmosphere during 11 days. Supernatants were collected and frozen at −70 °C to measure IgM, IgG and IgA. CD38 membrane expression was evaluated in cultured B cells.

2.3. Flow-cytometry

Purity of B cells was evaluated using a combination of anti-CD45FITC and anti-CD19PCy5 labelled monoclonal antibodies (both from Coulter Immunotech). To assess the maturation of 11 days cultured B cells, a combination of anti-CD45FITC, anti-CD19PCy5 and anti-CD38PE labelled monoclonal antibodies (all from Coulter Immunotech) was used. Differentiation of B cells was evaluated as the percentage of CD19$^+$ gated B cells in culture that developed a CD38$^+$ phenotype. Flow cytometry analysis was performed with an Epics FC500 using the CXP software (Beckman Coulter).

2.4. Immunoglobulin quantification

IgM, IgG and IgA were quantified in 11 days culture supernatants by BD Cytometric Bead Array Human Igs Flex Set (BD Biosciences) according to manufacturer instructions. The lower limits of detection were 0.06, 0.34 and 7.35 ng/ml for IgM, IgG and IgA, respectively. The assay was performed on an Epics FC500 flow cytometer and the data were analysed using the FCAP array software version 1.0.1 (BD Biosciences).

2.5. Statistical analysis

Statistical analysis was performed using the GraphPad Prism software (San Diego, California). Data are expressed as median