Human Immunology 71 (2010) 355–362

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

B-cell–T-cell activation and interaction in common variable immunodeficiency

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ARTICLE INFO

Article history:
Received 29 September 2009
Accepted 15 January 2010
Available online 19 February 2010

Keywords:
Activation
B cell
Common variable immunodeficiency
Cytokine
Proliferation
T cell

ABSTRACT

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders, characterized by hypogammaglobulinemia and normal or low numbers of B cells, which predispose patients to recurrent infections. Peripheral blood mononuclear cells from 19 patients with CVID, and age- and sex-matched controls, were subjected to an *in vitro* assay of B-cell–T-cell activation and interaction, using anti-immunoglobulin (Ig)-D conjugated to dextran (α-δ-dex), as a polyclonal T independent type 2 antigen mimic, with and without anti-CD3/anti-CD28, as polyclonal T-cell stimuli. Stimulation of lymphocytes with either anti-CD3 or anti-CD3 plus anti-CD28 induced T-cell activation and proliferation in CVID patients who were similar to age- and sex-matched controls, but B cells of patients were significantly less activated when peripheral blood mononuclear cells were stimulated with polyclonal T-cell agonists alone. Comparison of CD86 expression in the patients with matched controls revealed that patients had low B-cell activation in response to T-cell stimuli (bystander T-cell help). In conclusion, this sample of CVID patients exhibits a defect of T-cell "help" to B cells, and/or B-cell response to T-cell help.

1. Introduction

Common variable immunodeficiency (CVID) is a heterogeneous disease in the group of predominantly antibody deficiencies, which is defined by hypogammaglobulinemia and normal or low number of B cells, and characterized by increased susceptibility to recurrent bacterial infections, autoimmune disorders, and malignancies [1–6].

There is heterogeneity in the clinical manifestations and immunologic defects in CVID, which might reflect the heterogeneity of the underlying mechanisms [7,8]. Despite several years of investigation into the nature of CVID, the basic molecular defect(s) and pathogenesis of disease remains unknown [5,6]. However, several immunologic abnormalities leading to alteration of immunoglobulin concentrations have been identified. A number of defects in B-cell function, including impairment of upregulation of CD70 and CD86 in naive B cells, impaired somatic hypermutation, and defective antibody affinity maturation have been reported in CVID [9–11]. Defective antibody responses to polysaccharide and protein vaccines were also reported in many patients with CVID [12–14]. Moreover, several malfunctions in the T cells of CVID patients have been reported, including primary T-cell abnormalities [9,15,16], accelerated T-cell apoptosis [17], reduced generation of antigen-specific memory T cells [18], and abnormal cytokine production [19–21], which could be affected by cytokine gene polymorphisms [22–24].

Because there is heterogeneity of B-cell and T-cell defects in CVID, we considered that modeling B-cell and T-cell cognate interactions could help us to determine whether individuals with CVID have (a) defective B-cell activation and proliferation in response to the polyclonal T independent type 2 (TI-2) antigen, (b) defective T-cell activation and proliferation in response to polyclonal T-cell stimuli in the presence or absence of stimulated B cells, (c) defective B-cell activation and proliferation in response to activated T cells in the presence or absence of a polyclonal TI-2 antigen.

2. Subjects and methods

2.1. Subjects

Nineteen patients with CVID (13 male and six female), with age more than 14 years, and 18 age- and sex-matched controls (12 male

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0198-8859/10/$32.00 - see front matter © 2010 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.humimm.2010.01.019

and six female) were enrolled into the study. Characteristics of the patients are presented in the Table 1. All patients fulfilled international criteria for the diagnosis of CVID, including low serum levels of at least 2 immunoglobulins (Ig) (IgG, IgA, IgM) by two standard deviations from the normal mean values for age and genetic exclusion of other diseases associated with well-defined single gene defects. All patients gave informed consent and the project was approved by the UK National Research Ethics Service (04/S0501/34) and the Immunology, Asthma and Allergy Research Institute Ethics Committee (412/88/204).

2.2. Study design

An assay of B-cell and T-cell interaction was set up using anti-IgD conjugated to dextran (α-δ-dex), as a TI-2 antigen mimic, with and without anti-CD3/anti-CD28, as polyclonal T-cell stimuli. The effects of combinations of these stimuli on B-cell and T-cell activation and proliferation were assessed. The assay was broadly designed as reported previously by Foster *et al.* [25].

The study was designed in three steps:

A. Assessment of B-cell proliferation and activation in response to the polyclonal mimic of TI-2 antigens (α-δ-dex).
B. Assessment of T-cell responsiveness to polyclonal stimuli designed to mimic signal 1 (through the antigen-specific T-cell receptor) *via* anti-CD3, and signal 2 (through the activated antigen-presenting cell) *via* anti-CD28, which are required for maximal T-cell activation and proliferation. It is also possible to assess T-cell proliferation and activation that occur when activated B cells stimulate interacting T cells, by providing signal 1 to the T cells *via* anti-CD3 and relying upon α-δ-dex–stimulated B cells to provide signal 2 through expression of CD86 or other costimulatory molecules.
C. Assessment of the ability of T cells to provide help to B cells inducing activation and/or proliferation in the presence or absence of α-δ-dex.

All parts of the assay were performed simultaneously on patients and their age- and sex-matched controls.

2.3. Preparation of blood

Approximately 15 ml blood was obtained from the subjects. In the patients, heparinized blood was taken immediately before the next immunoglobulin infusion. The blood was diluted 1:2 with phosphate-buffered saline (PBS) and the diluted blood was carefully layered onto half the volume of lympholyte (Lympholyte-H, Cedarlane Laboratories Ltd, Luxembourg, The Netherlands). The cells were centrifuged at 400*g*, at 20 °C for 35 minutes; the lymphocyte layer was removed, and diluted again with chilled PBS. The cells were centrifuged at 600*g*, at 4 °C for 15 minutes. Then, the supernatant was discarded and the cells were washed three times.

The cells were resuspended in 1 ml of RPMI (RPMI 1640 + L-Glutamine, Gibco, Invitrogen, United Kingdom) at a concentration of 1×10^7 cells/ml medium (RPMI containing 10% autologous human plasma). Some cells were set aside unstained and the remainder were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE), which enables assessment of proliferation of the cells by flow cytometry.

Table 1
Characteristics of the patients with CVID investigated in this study

Patient number	Sex	Study age (y)	Duration from diagnosis/treatment	Clinical manifestations	Complications	Serum Ig level (mg/dl)			Lymphocyte subsets (%)			
						IgG	IgM	IgA	CD19	CD3	CD4	CD8
P1	Female	54	2 y	GI, LRT, OA	No	170	0	0	10.10	80.60	27.50	55.60
P2	Male	51	10 y 4 m	GI, LRT, URT	Bronchiectasis, malignancy	50	10	0	7.02	70.40	23.95	40.14
P3	Male	49	6 y	GI, LRT, UT	Bronchiectasis	20	26	0	11.00	54.00	31.00	22.50
P4	Female	30	14 y 11 m	GI, LRT, URT	No	50	10	0	11.19	79.25	40.42	35.23
P5	Male	28	9 y	GI, LRT, URT	Bronchiectasis, splenomegaly	50	0	0	12.00	85.00	57.00	52.00
P6	Female	26	23 y 3 m	GI, LRT, URT, OA, MC	Bronchiectasis	100	10	5	2.10	88.60	66.20	19.00
P7	Male	24	17 y 3 m	GI, LRT, URT, UT	Bronchiectasis, splenomegaly, autoimmunity	100	0	0	8.50	62.20	24.70	35.40
P8	Male	24	15 y	LRT, URT, CNS	Bronchiectasis, splenomegaly, autoimmunity	50	10	5	10.00	65.00	35.00	32.00
P9	Female	20	7 y 6 m	GI, LRT, URT, OA, MC	Bronchiectasis	100	0	0	8.32	85.25	23.42	49.95
P10	Female	20	15 y 6 m	GI, LRT, URT, OA	Splenomegaly, autoimmunity, malignancy	410	20	10	15.11	78.11	38.33	35.08
P11	Male	19	14 y 1 m	GI, LRT, URT, MC	Bronchiectasis, splenomegaly, autoimmunity	360	42	10	2.16	62.67	6.82	50.35
P12	Male	18	4 y 8 m	LRT, URT, OA, MC	No	20	140	6	17.15	71.00	36.07	24.68
P13	Male	18	6 y 4 m	LRT, URT, MC	Bronchiectasis, splenomegaly, malignancy	100	29	10	31.4	65.00	31.70	34.20
P14	Male	18	6 y 10 m	GI, LRT, URT, OA	Splenomegaly	100	10	5	22.91	67.59	20.85	43.85
P15	Male	16	5 y 8 m	GI, URT	Splenomegaly, autoimmunity	270	35	27	10.00	63.00	29.00	23.00
P16	Male	16	11 y	GI, LRT, OA	Bronchiectasis, splenomegaly	100	20	5	3.85	88.27	31.85	55.48
P17	Female	15	7 y 10 m	GI, LRT, URT, UT, MC	No	20	10	5	24.90	66.38	35.23	31.29
P18	Male	15	6 y 11 m	LRT, URT	No	470	10	5	7.50	80.90	43.10	34.60
P19	Male	15	11 y 8 m	GI, URT	No	310	48	10	21.90	66.70	37.40	26.40

y = year; m = month; GI = gastrointestinal tract infection (diarrhea); URT = upper respiratory tract infection (sinusitis, otitis media); LRT = lower respiratory tract infection (pneumonia); UT = urinary tract infection (pyelonephritis); CNS = central nervous system infection (meningitis); OA = osteo-articular infection (osteomyelitis, arthritis); MC = mucocutaneous manifestation (abscess, candidiasis).

2.4. CFSE staining

The cells were incubated at 1×10^7 cells/ml in 2 μM CFSE diluted in medium; the cells were incubated at 37 °C with 5% CO_2 for 10 minutes in the dark. After quenching the cells with equal volumes of autologous serum, the cells were incubated at room temperature in the dark for 10 more minutes. The cells were washed with medium three times (centrifuged at 400*g*, at 20 °C for 8 minutes) and were finally resuspended at a concentration of 1×10^7/ml medium, before 100 μl cells plus 400 μl medium (final concentration of 2×10^6 cells/ml) were plated out onto the plates.

2.5. Cell stimulation

Sterile 24-well, tissue culture plates (Nunc) were used for the assay for each subject. Wells were precoated with anti-CD3 at 0.1 μg/ml (CD3, Mouse Anti-Human, Purified; Invitrogen) each and other wells were precoated with anti-CD3$^+$ anti-CD28 at 0.5 μg/ml

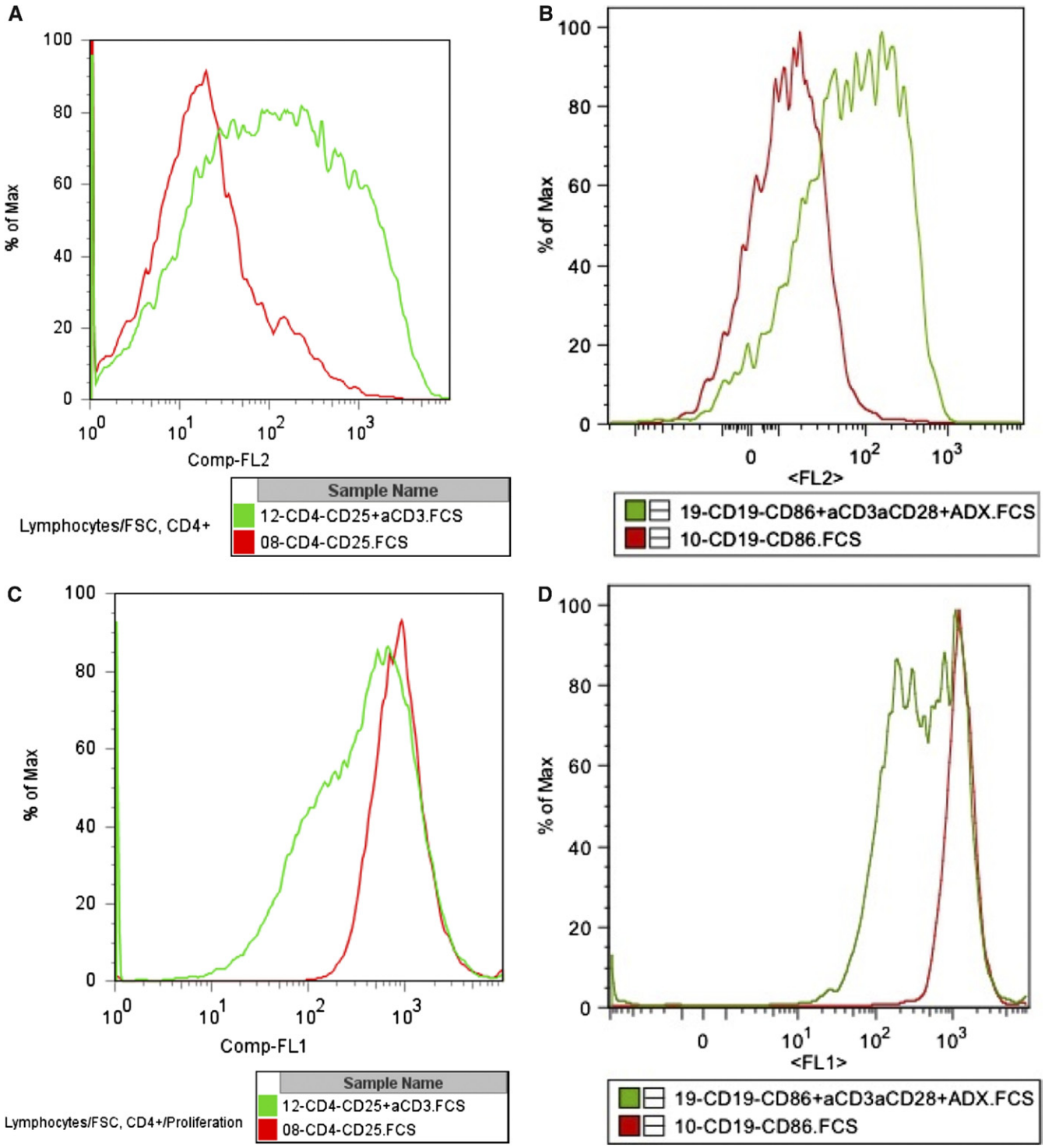

Fig. 1. Expression of activation markers and proliferation assay on the gated cells. In all cases, the red histogram is the unstimulated control. (A) Expression of CD25 by CD4-positive T cells, which shows an increase after activation by anti-CD3. (B) Expression of CD86 after activation by anti-CD3 + anti-CD28 + α-δ-dex. (C) Proliferation of T cells after stimulation by anti-CD3. (D) B-cell proliferation after stimulation by anti-CD3 + anti-CD28 + α-δ-dex.

(CD28, Mouse Anti-Human, Purified; Invitrogen). Wells were washed 24 hours after precoating.

α-δ-dex consists of high-molecular-weight dextran polysaccharide carrier molecules, which are coupled to polyclonal anti-IgD antibodies that can induce a multivalent stimulus to B cells [26,27]. The concentration of α-δ-dex [26,28] used was 1 μg/ml.

For each patient or control, cells were stimulated with α-δ-dex, anti-CD3, anti-CD3$^+$ α-δ-dex, anti-CD3$^+$ antiCD28, anti-CD3$^+$ anti-CD28$^+$ α-δ-dex, or media alone (Unstimulated). For each patient or control, cells were left unstimulated or stimulated with either anti-CD3 or anti-CD3$^+$ anti-CD28; all in the presence or absence of α-δ-dex.

Cells were incubated for 4 days in a humidified atmosphere at 37 °C, 5% CO_2 before harvesting.

2.6. Cell harvesting

On day 4, the cells were harvested by vigorous pipetting into microtiter tubes for flow cytometry.

The cells were centrifuged at 400*g*, at 4 °C for 5 minutes and the supernatants were removed and stored at −20 °C, for later cytokine analysis.

The cell pellet was resuspended in 1 ml cold fluorescence-activated cell sorting buffer (1% bovine serum albumin in PBS),

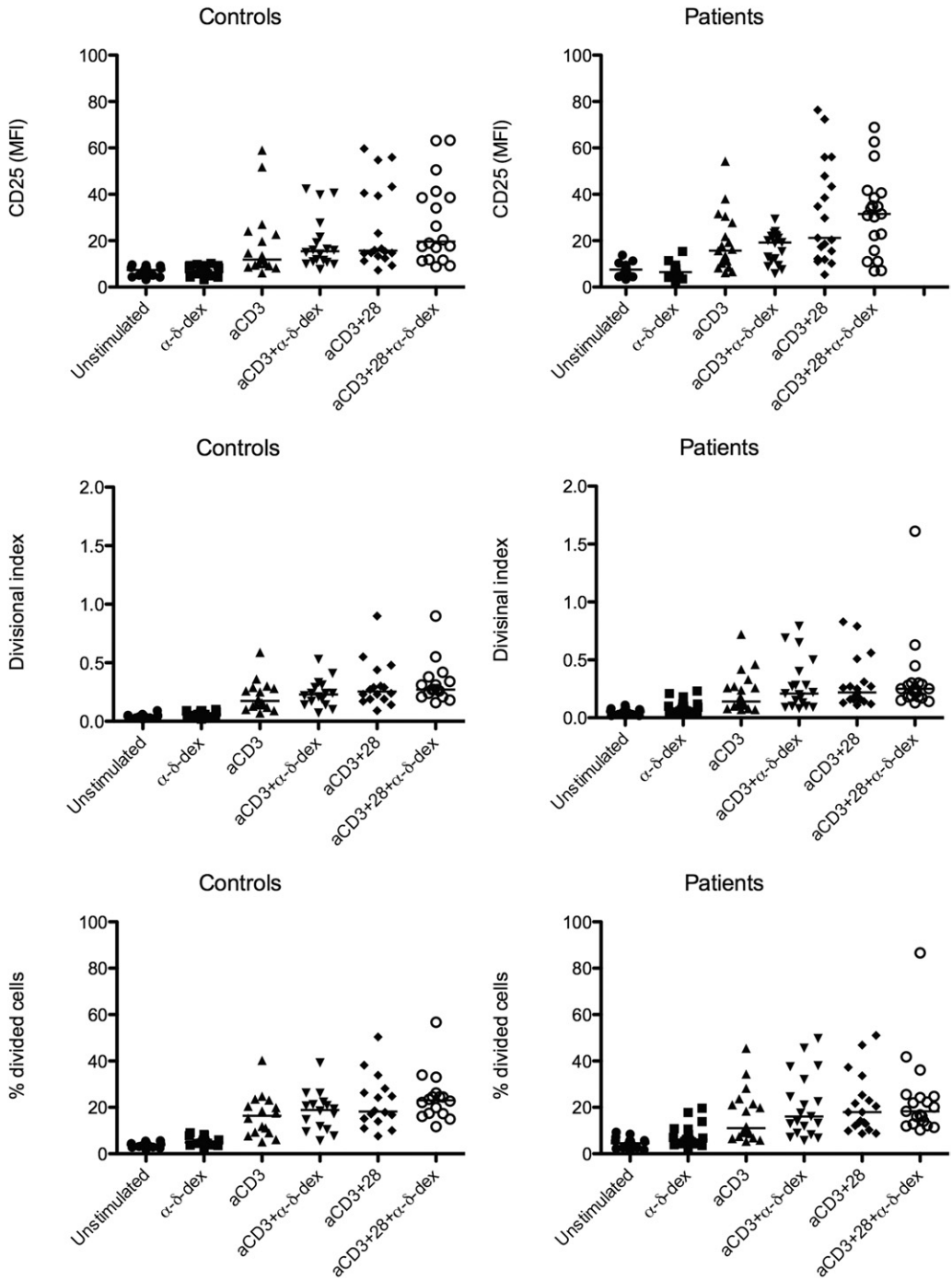

Fig. 2. Proliferation (Divisional index and % divided cells) and CD25 expression (Median fluorescence index) of CD4+ cells in both CVID patients and matched controls. PBMCs were activated with plate-bound anti-CD3 ± anti-CD28 and/or the B-cell stimulator α-δ-dex. Line denotes median.

and washed three times with fluorescence-activated cell sorting buffer.

2.7. Flow cytometry

The following antibodies were obtained from Invitrogen: CD4, Mouse Anti-Human (PE-Cy5.5), CD19, Mouse Anti-Human (PE-Cy5.5), CD86, Mouse Anti-Human (R-PE), CD25, Mouse Anti-Human (R-PE), Mouse IgG1 (R-PE) as of isotype control for $CD25^{-}$R-PE and $CD86^{-}$R-PE, Mouse IgG1 (PE-Cy5.5) as of isotype control for $CD19^{-}$PE-Cy5.5, Mouse IgG2a (PE-Cy5.5) as of isotype control for $CD4^{-}$PE-Cy5.5.

Peripheral mononuclear cells were labeled with specific cell markers for T helper ($CD4^{+}$) and B ($CD19^{+}$) cells plus activation markers CD25 and CD86, respectively (Fig. 1A, B). Then, the activation of T and B lymphocytes was assessed measuring the upregulation of CD25 or CD86 in these cells, respectively, and expressed as mean fluorescence intensity.

Proliferation of T and B cells was measured using a CFSE-based assay on the cells gated for CD4 or CD19 expression, respectively [25,29].

Flow cytometry was performed using a Partec flow cytometer (Partec GmbH, Munster, Germany) and FlowJo software; version 7.2.5 (Tree Star, Ashland, OR) was used for analysis of flow cytometry data.

2.8. Cytokine assay

Measurement of interleukin (IL)-10 and interferon (IFN)-γ concentrations was performed on the supernatants of the unstimu-

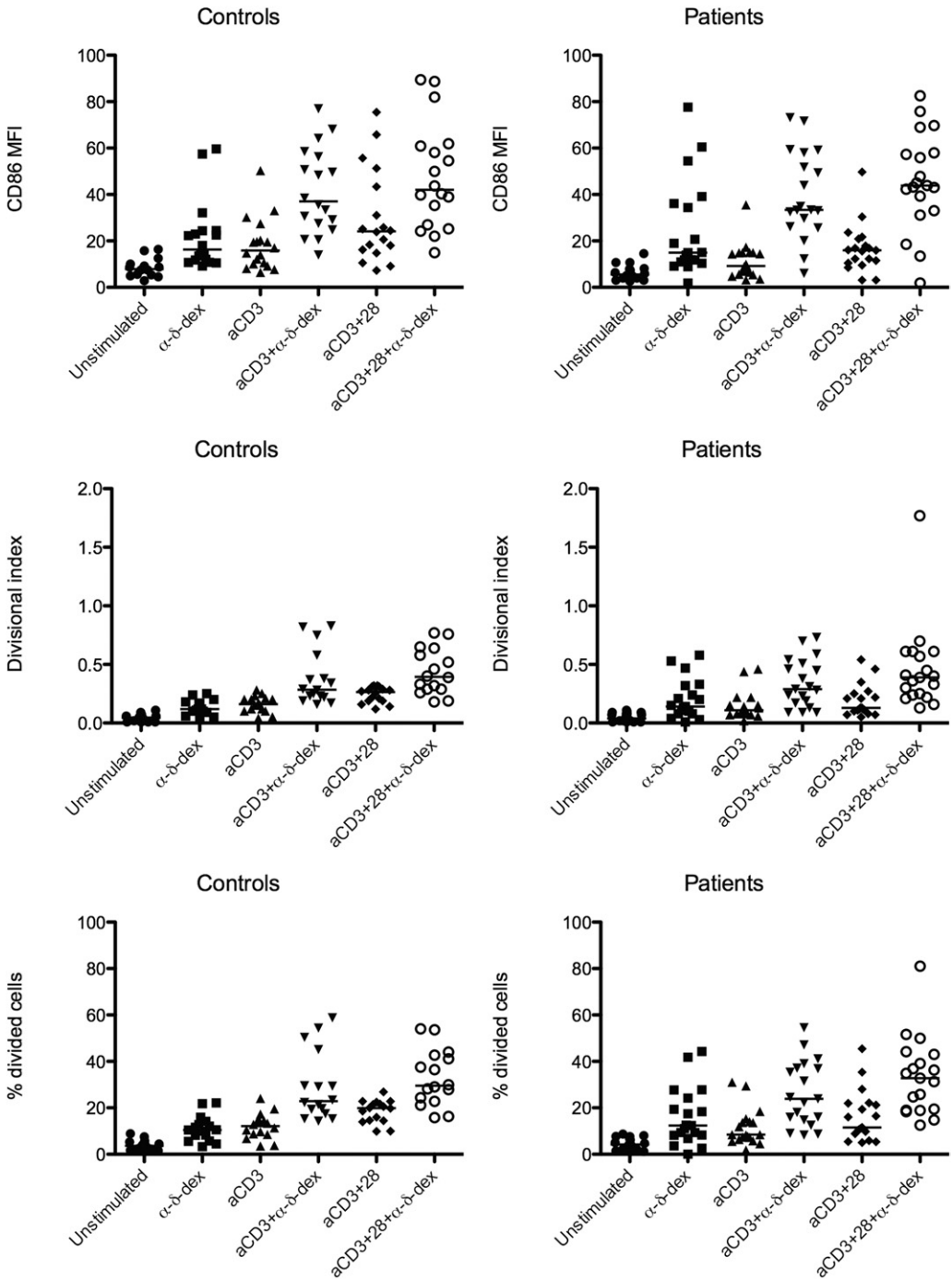

Fig. 3. Proliferation (Divisional index and % divided cells) and CD86 expression (Median fluorescence index) of CD19+ cells in both CVID patients and matched controls. PBMCs were activated with plate-bound anti-CD3 ± anti-CD28 and/or the B-cell stimulator α-δ-dex. Line denotes median.

lated and stimulated cells with α-δ-dex, in the presence or absence of anti-CD3/anti-CD28, by enzyme-linked immunosorbent assay (R&D Systems, United Kingdom).

2.9. Statistical analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS) software, version 16.0 (SPSS, Chicago, IL). T-cell and B-cell activation were expressed as mean fluorescence intensity of CD25 and CD86 expression on $CD4^+$ and $CD19^+$ cells, respectively. The data were found to have a log-normal distribution; so data were logarithmically transformed to base 10 (Log_{10}), before statistical analysis, to give a Gaussian distribution (as determined by analysis of skewness and a nonsignificant D'Agostino and Pearson omnibus normality test result) and allow the use of more powerful parametric tests such as analysis of variance (ANOVA). Statistical comparison between patients and controls with the differing stimuli was performed by two-way ANOVA with Bonferroni post test. Comparisons between unstimulated and stimulated cells were performed using one-way ANOVA with Bonferroni selected pairs post test.

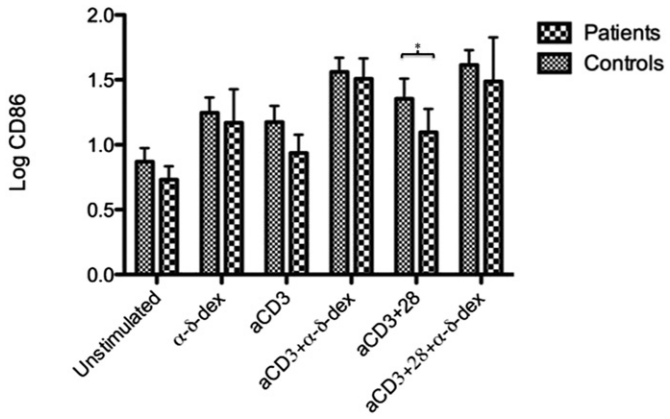

Fig. 4. Log_{10} expression of CD86 by CD19-positive B cells. PBMCs were activated with plate-bound anti-CD3 + anti-CD28 and/or α-δ-dex. CD86 expression induced by anti-CD3 + anti-CD28 was significantly reduced in patients when compaired to controls. Two-way ANOVA with Bonferroni post test.

3. Results

Example histograms of activation and proliferation are given in Fig. 1. Raw data relating to proliferation and activation of T cells (Fig. 2) and B cells (Fig. 3) under all experimental conditions in patients and controls are shown as scatter plots.

3.1. T-cell activation

Log_{10} median fluorescence intensity of CD25 significantly increased in both groups of patients and controls in response to anti-CD3 (0.839 ± 0.16 vs. 1.2 ± 0.26, $p \leq 0.001$ in patients; and 0.82 ± 0.14 vs. 1.17 ± 0.27, $p \leq 0.001$ in controls). $CD4^+$ T cells were further efficiently activated by costimulation of anti-CD3 and anti-CD28 (0.839 ± 0.16 vs. 1.39 ± 0.32, $p \leq 0.001$ in patients; and 0.82 ± 0.14 vs. 1.31 ± 0.29, $p \leq 0.001$ in controls).

Comparisons of CD25 expression induced by different stimuli showed that there was no significant difference in the response of patient T cells to any of the stimuli, compared with the controls.

3.2. T-cell proliferation

Division index (average number of cell divisions that the responding cells underwent), % divided (percentage of the cells of the original sample which divided), were calculated using FlowJo software.

Division index and percentage of divided $CD4^+$ T cells were significantly increased in both patients and controls in response to anti-CD3. T cells were further appropriately proliferated by costimulation with anti-CD3 and anti-CD28 (Table 2).

Comparisons of division index and percent divided showed that there was no significant difference in the ability of patient T cells to respond to either stimulus, compared with the controls.

3.3. B-cell activation

B cells were activated efficiently in response to α-δ-dex in both groups of patients and controls. Log_{10} median fluorescence intensity of CD86 was significantly increased from 0.75 ± 0.12 (unstimulated) to 1.24 ± 0.37 (stimulated with α-δ-dex) in the patients ($p = 0.001$) and from 0.88 ± 0.18 (unstimulated) to 1.26 ± 0.24 (stimulated with α-δ-dex) in the controls ($p = 0.001$). In addition, B cells were also activated by T-cell stimuli (anti-CD3 with/without anti-CD28) and this proved synergistic with α-δ-dex in both groups (Fig. 3).

Although there were no significant differences in the response of patient B cells to α-δ-dex compared with the controls, patient B cells were significantly less activated than the controls in the presence T-cell stimuli alone (Fig. 4).

3.4. B-cell proliferation

Analysis of division index and percentage of divided B cells revealed significant increases in B-cell proliferation in both groups of patients and controls in response to α-δ-dex. B cells also proliferated after stimulation with anti-CD3 with/without anti-CD28 (Table 3).

Costimulation of α-δ-dex with T-cell stimuli leads to significant increases in division index and percentage of divided B cells, compared with T-cell stimuli alone, in both patients and controls. In view of the normal B-cell proliferation in response to α-δ-dex alone, it was concluded that some patients exhibit an aberrant response to α-δ-dex stimulation when B cells were costimulated with T-cell stimuli.

3.5. Cytokine production

IFN-γ production was significantly increased in both groups of patients and controls in response to anti-CD3 (19.68 ± 7.19 vs. 2129.72 ± 1097.48 pg/ml, $p < 0.001$ in patients; and 23.91 ± 8.32 vs. 2240.33 ± 895.34 pg/ml, $p < 0.001$ in controls). IFN-γ production was further efficiently increased by costimulation with anti-CD3 and anti-CD28 (Table 4). Comparison of cytokine production between different T-cell stimuli indicated that there was a signifi-

Table 2
Log_{10} division index and percentage of divided CD4+ T cells in response to T-cell stimuli in CVID patients and controls

	CD4+ T cells (Mean ± SD)		*p* Value	CD4+ T cells (Mean ± SD)		*p* Value
	Unstimulated	Stimulated with anti-CD3		Unstimulated	Stimulated with anti-CD3 + anti-CD28	
Patients						
Division index	−1.32 ± 0.22	−0.76 ± 0.3	≤0.001	−1.32 ± 0.22	−0.61 ± 0.26	≤0.001
% divided	0.6 ± 0.22	1.1 ± 0.27	≤0.001	0.6 ± 0.22	1.25 ± 0.23	≤0.001
Controls						
Division index	−1.40 ± 0.15	−0.73 ± 0.25	≤0.001	−1.40 ± 0.15	−0.57 ± 0.24	≤0.001
% divided	0.55 ± 0.12	1.15 ± 0.24	≤0.001	0.55 ± 0.12	1.29 ± 0.22	≤0.001

Table 3
Log_{10} division index and percentage of divided B cells in response to T-cell stimuli and costimulation of α-δ-dex in CVID patients and controls

	CD19+ B cells (Mean ± SD)		*p* Value	CD19+ B cells (Mean ± SD)		*p* Value	CD19+ B cells (Mean ± SD)
	Stimulated with anti-CD3*	Stimulated with anti-CD3 + α-δ-dex		Stimulated with anti-CD3 + anti-CD28[a]	Stimulated with anti-CD3 + anti-CD28 + α-δ-dex		Stimulated with α-δ-dex
Patients							
Division index	−0.94 ± 0.31	−0.55 ± 0.30	≤0.01	−0.81 ± 0.28	−0.42 ± 0.26	≤0.01	−0.86 ± 0.44
% divided	0.97 ± 0.29	1.35 ± 0.25	≤0.001	1.12 ± 0.28	1.47 ± 0.20	≤0.001	1.11 ± 0.33
Controls							
Division index	−0.84 ± 0.23	−0.48 ± 0.23	≤0.001	−0.63 ± 0.13	−0.39 ± 0.2	≤0.05	−0.93 ± 0.2
% divided	1.03 ± 0.23	1.41 ± 0.20	≤0.001	1.25 ± 0.17	1.48 ± 0.16	≤0.01	0.99 ± 0.22

[a]All indices of stimulated cells with α-δ-dex, anti-CD3 or anti-CD3 + anti-CD28 were significantly increased, compared with unstimulated cells ($p \leq 0.001$).

cant increase in IFN-γ production by the cells that were stimulated with anti-CD3 and anti-CD28, compared with the cells that were stimulated with anti-CD3 ($p = 0.006$ in patients and $p = 0.004$ in controls). However, α-δ-dex had no effect on cytokine production. Comparisons of mean fold changes in IFN-γ secretion induced by different stimuli showed that there was not any significant difference in cytokine production of the patients, compared with the controls.

IL-10 production significantly increased in both groups of patients and controls in response to either anti-CD3 alone ($p = 0.011$) or costimulation of anti-CD3 and anti-CD28 ($p = 0.028$ for patients and $p = 0.008$ for controls) (Table 4); However, α-δ-dex again had no effect on this cytokine production.

4. Discussion

B-cell activation and differentiation depends on B-cell–T-cell interaction. Modeling of B-cell and T-cell cognate interaction was done to evaluate differences between CVID patients and controls in B-cell and T-cell proliferation and activation in response to polyclonal T-cell stimuli and/or a polyclonal B-cell stimulus (a TI-2 antigen mimic). T-cell responsiveness was designed to mimic signal 1 (through the antigen-specific T-cell receptor) and signal 2 (through the activated antigen-presenting cell) [25], which are both required for maximal T-cell activation and proliferation [4]. B-cell responsiveness in response to the polyclonal mimic of TI-2 antigens was assessed and the effect of T-cell help on B cells was assessed both in the presence and absence of the mimic TI-2 antigen. B-cell and T-cell activation in response to these signals was assessed by measuring expression of the activation marker CD86 on gated $CD19^+$ B cells and the activation marker CD25 on gated $CD4^+$ T cells, respectively. Proliferation was assessed using the CFSE dilution assay [25].

Stimulation of lymphocytes with either anti-CD3 or anti-CD3 plus anti-CD28 revealed T-cell activation and proliferation in CVID patients similar to the controls. T cells perform their B helper function through either membrane costimulatory molecules or production of cytokines. However, there are some controversies on expression and function of costimulatory molecules on T cells of CVID patients [30–32]. Pons *et al.* evaluated the expression and upregulation of costimulatory molecules (CD28, CD40L/CD154, and CTLA-4/CD152) in purified T cells of patients with CVID patients in response to either anti-CD3 or anti-$CD3^+$ anti-CD28. They showed that stimulated T cells of patients expressed normal levels of these costimulatory molecules [30], which agrees with our findings on T-cell activation and proliferation in the patients. However, B cells of the patients in the current study were significantly less activated in wells containing only T-cell stimuli, indicating lower levels of T cell "help." A subgroup of CVID patients, with normal numbers of mature surface(s) IgM/sIgD-positive circulating B cells, was previously defined [33,34]. Denz *et al.* showed that these patients have decreased expression of CD86 following *in vitro* activation of PBMC or purified B cells with anti-IgM plus IL-2; thus, their B cells are unable to cooperate with T cells [33]. Further evaluation by Groth *et al.* revealed significantly lower expression of the activation markers CD25 and CD86 in naive B cells of the CVID patients; thus, an intrinsic signaling or expression defect for CD70/CD86 at the level of naive B cells was inferred in a subgroup of CVID patients [34]. Inability of $CD4^+$ T cells to provide T-cell help for B cells could lead to low serum immunoglobulin concentrations of immunoglobulin isotypes in a group of patients with CVID [35]. The inability of B cells of some patients to express CD86 in response to T-cell help did not appear to be an intrinsic defect in CD86 expression, as expression of CD86 in response to α-δ-dex was normal.

In addition to direct cell-cell interactions, B helper function of T cells can occur through cytokines that interact with their receptors on the target cells [30]. The production of IFN-γ and IL-10 was significantly increased in both groups of patients and controls in response to either anti-CD3 or anti-CD3/anti-CD28; however, there was no significant difference between the patients and the controls. Similar findings were described by Pons *et al.*, in which T cells of patients produced similar amounts of cytokines in comparison with controls, when stimulated with optimal doses of anti-CD3 or suboptimal doses of anti-CD3 plus anti-CD28 [30].

CVID is a heterogenous group of disorders. We have identified a subset of patients who likely have a defect in the ability of their T cells to deliver "help" to B cells, or in the ability of their B cells to

Table 4
Cytokine production in response to T-cell stimuli in CVID patients and controls

	IFN-γ pg/ml (Mean ± SD)		*p* Value[a]	IFN-γ pg/ml (Mean ± SD)		*p* Value[a]
	Unstimulated	Stimulated with anti-CD3		Unstimulated	Stimulated with anti-CD3 + anti-CD28	
Patients	19.68 ± 7.19	2129.72 ± 1097.48	<0.001	19.68 ± 7.19	2687.40 ± 1196.74	<0.001
Controls	23.91 ± 8.32	2240.33 ± 895.34	<0.001	23.91 ± 8.32	2633.78 ± 993.68	<0.001
	IL-10 pg/ml (Median)		*p* Value[b]	IL-10 pg/ml (Median)		*p* Value[b]
	Unstimulated	Stimulated with anti-CD3		Unstimulated	Stimulated with anti-CD3 + anti-CD28	
Patients	180.1	466.1	0.011	180.1	533.0	0.028
Controls	190.6	254.1	0.011	190.6	466.1	0.008

[a]Paired-samples t test.
[b]Wilcoxon Signed Rank test.

receive T-cell help. Further work will be required to elucidate the molecular mechanisms behind such a defect or defects.

Acknowledgments

This research was supported by grants from Immunology, Asthma and Allergy Research Institute and Tehran University of Medical Sciences and Health Services (87-04-30-8389), the UK Meningitis Research Foundation (Grant 0604.0), and the Binney Fund (Sheffield Hospitals Charitable Funds). The authors are very grateful for all colleagues in the Department of Allergy and Clinical Immunology of Children's Medical Center and the Immunology, Asthma and Allergy Research Institute for their kind help and advice in the laboratory and also to all the patients and their families for their kind collaboration in this study. We thank Mohsen Siavashi and Reza Khalili for their contribution in collecting the samples from the subjects. We also thank Susan Newton of the University of Sheffield Medical School Flow Cytometry Core Facility for help and advice.

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