



Research paper

Evaluation of class switch recombination in B lymphocytes of patients with common variable immunodeficiency

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ABSTRACT

Common variable immunodeficiency (CVID) is a heterogeneous group of disorders characterized by hypogammaglobulinemia and recurrent bacterial infections. Impaired antibody production in these patients is due to defect in B-cell differentiation into specific plasma cells. Class switch recombination (CSR), which plays a critical role in the production of different immunoglobulin isotypes, may be defective in a group of CVID patients. The aim of this study was to investigate the CSR process in B cells of CVID patients, by evaluating the expression of IgE mRNA and production of its protein in an IgE inductive medium. Peripheral blood mononuclear cells (PBMCs) from 29 CVID patients and 21 healthy controls were isolated and cultured in the presence of rhIL-4 and CD40L. IgE mRNA and IgE protein were measured by RT-PCR and ELISA techniques, respectively. Normal production of IgE mRNA was recorded in 23 out of 29 patients (79.31%) as well as all controls; while the remaining 6 patients (20.69%) were unable to express IgE mRNA indicating a defect in CSR. PBMCs of 16 out of 29 patients (55.2%) could not produce normal amounts of IgE compared with controls, after being stimulated by IL-4 and CD40L. Post B cell stimulation IgE production was impaired in about half of studied CVID patients. Defects in processes occur following the CSR process such as IgE mRNA transcription, protein production, and secretion can be the causative mechanism of CVID in patients with normal mRNA expression of the immunoglobulin but impaired protein production. Determination of these defects can help to clarify the various underlying mechanisms responsible for the development of CVID.

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1. Introduction

Common variable immune deficiency (CVID) is a heterogeneous disorder characterized by impaired production of specific antibodies against special foreign antigens. Hypogammaglobulinemia, including reduced levels of

Immunoglobulin (Ig) G, A and/or M, predisposes patients with CVID to recurrent infections (Aghamohammadi et al., 2005; Cunningham-Rundles and Bodian, 1999). Divergent clinical findings and immune dysregulation in CVID patients can reflect the heterogeneity of the mechanisms leading to this disorder (Di Renzo et al., 2004). Defective antibody production in CVID is result of a failure in differentiation of B cells into immunoglobulin-secreting cells (Nonoyama et al., 1993).

Class switch recombination (CSR) has a critical role in late stages of Ig production. It occurs in mature B cells after antigen engagement and requires co-stimulatory signals from CD4+ T cells such as CD40 ligand (CD40L) and various cytokines like IL-4 and IL-10 to produce different isotypes of antibody

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(Kracker and Durandy, 2011). CSR deficiencies lead to normal or increased IgM production, and reduced or absent Ig isotype switching to produce Ig G, Ig A, or Ig E (Durandy et al., 2007).

There are some reported defects in the function of B cells in CVID patients. They include incompetence in early and late B cell differentiation, impaired expression of CD27, CD86, and CD70, impaired antibody affinity maturation and somatic hyper-mutation, impaired signaling, and deficiency of memory B cells (Bayry et al., 2005; Bonhomme et al., 2000; Levy et al., 1998). So far various defects in the following genes such as *BAFF-R*, *CD19*, *TACI*, *ICOS*, and *MSH5* have been reported (Castigli et al., 2005; Salzer et al., 2005; Sekine et al., 2007; van Zelm et al., 2006). These known genetic defects involve only about 25% of CVID patients, while other mechanisms involved in the etiology in this disorder are not clear. Therefore a clarified causing mechanism is yet to be determined (Blanco-Quiros et al., 2006).

There are some investigations on the CSR process and Ig production in CVID patients after stimulation and activation by various required cytokines such as IL-2, IL-4, and IL-10 in combination with anti-CD40 (Eisenstein et al., 1994; Nonoyama et al., 1993; Pastorelli et al., 1990). However, different results on B cell response to stimulation, subsequent differentiation, and Ig production were reported. Combination of CD40-L and IL-4 directly stimulates the proliferation of B cells and IgE isotype switching (Shapira et al., 1992). Therefore this method of stimulation can exclusively evaluate the CSR function of B cells in CVID patients. The aim of this study is to investigate the CSR process in B cells of CVID patients by evaluating the production of IgE mRNA and its protein to improve our understanding of defective molecular and cellular mechanisms responsible for the etiology of CVID.

2. Materials and methods

2.1. Patients

Twenty-nine CVID patients (24 male and 5 female) and a control group of 21 age and sex matched healthy individuals were enrolled in this study. On some occasions, two patients were matched with one individual in the control group. The diagnosis of CVID was made in all of the patients by using standard criteria of the European Society for Immune Deficiency (ESID) and Pan American Group for Immunodeficiency (PAGID) (Chapel, 1994). According to these criteria, the diagnosis of CVID was confirmed when patient's age was higher than 4 years and serum levels of IgG and at least one of serum IgA or IgM were lower than 2 standard deviations from normal mean range for age and sex matched individuals. All patients also had evidence of defect in T-cell independent immune response (as measured by lacking isohemagglutinins) and/or defect of T-cell dependent immune response (as measured by lacking response to vaccination). The other causes of hypogammaglobulinemia also were excluded in these patients. All patients received monthly intravenous immunoglobulin (IVIG) and blood samples were prepared before taking their IVIG. None of the patients had active infection at the time of study. The process of this study was approved by the ethic committee of the Tehran University of Medical Sciences and informed consent was taken from all subjects.

2.2. Blood cell culture

A 5-ml blood sample was taken from each patient or healthy donor. Peripheral blood mononuclear cells (PBMCs) were isolated from the samples using Ficoll–Paque density gradient centrifugation. Separated PBMCs were then washed twice with Phosphate-Buffered Saline (PBS). Isolated and washed PBMCs (1×10^5 cells) were cultured in Iscove's Modified Dulbecco's Medium (IMDM) (Sigma Aldrich, Germany) containing 10% fetal bovine serum (FBS), 1% sodium pyruvate and 1% non-essential amino acids (all prepared from Invitrogen, Germany) at 37 °C and 5% CO₂.

To create the suitable environment for class switching of B lymphocyte to IgE, 400 ng/ml rh-IL4 and 200 IU/ml rh-CD40L (both prepared from Invitrogen, Germany) were added to culture mediums. Moreover, 100 U/ml penicillin and 100 µg/ml of streptomycin were also added to culture medium in order to prevent bacterial contamination (Armitage et al., 1993; Shapira et al., 1992).

After 5 and 12 days of culturing the expression of IgE mRNA and protein was determined in cultured cells and their supernatants respectively.

2.3. Evaluation of IgE mRNA expression

The mRNA of cultured PBMCs was extracted after 5 days by the use of 0.5 ml Guanidinium thiocyanate-phenol-chloroform (TRIzol, Gibco-BRL, USA) at 4 °C. The contaminated DNA was removed with DNase 1 (Gibco-BRL, USA). This temperature was reached by using crushed ice. For determination of mRNA purity the ratio of optical density (OD) at 260 nm to the OD at 280 nm was measured by BioPhotometer (Roche, Germany). This ratio was always more than 1.8. The expression of *Actin* gene mRNA was also determined as internal control.

Real time polymerase chain reaction (RT-PCR) was performed using cDNA Synthesis Kits (Fermentas, Canada). Simply, after mRNA extraction, cDNA (for *IgE* and *Actin* genes) were produced using Oligo (dT) primer and reverse transcriptase enzyme. Specific primers for each gene were designed using the information on GenBank nucleotide sequence database and their characteristics were evaluated using Oligo V6 software (Molecular Biology Insights, Inc., Cascade, CO). Oligonucleotide sequences of these primers are shown in Table 1. Finally, PCR method was performed on produced cDNAs using Master Mix Kit (Fermentas, Canada).

2.4. Evaluation of B cell proliferation

Isolated and washed PBMCs from patients and controls (1×10^6 cells) were suspended in Roswell Park Memorial Institute medium (RPMI 1640) (Gibco, Germany) supplemented with 10% fetal bovine serum (FBS), 1% sodium pyruvate and 1%

Table 1
Primer sequences for *Actin* and *IgE*.

Gene	Primer sequence
<i>Actin</i>	Forward: 5'-TACCACTGGCATCGTGATGGACT-3'
	Reverse: 5'-TCCTTCTGCATCCTGTGCGCAAT-3'
<i>IgE</i>	Forward: 5'-GACACGGCCGTGTATTACTG-3'
	Reverse: 5'ACGGAGGTGGCATTGGAGGAAATGT-3'

non-specific amino acids (all prepared from Invitrogen, Germany), to be stimulated by 200 IU/ml rh-IL4 and 400 ng/ml rh-CD40L. In the next step, 2×10^5 PBMCs were added to each well of the 96-well round bottom microculture plates (Falcon, USA). All tests were done in triplicate and plates were incubated at 37 °C with 5% CO₂ for 102 h. Afterward, 1 µCi [³H] thymidine (Amersham Pharmacia, Sweden) were added per well for the last 16 h before harvesting on glass fiber filters (Whatman, USA) and liquid scintillation counting. The results were presented as counts per minute (CPM) and stimulation index (SI). Stimulation index of lymphocytes was calculated according to the formula:

$$SI = \frac{CPM_S}{CPM_C}$$

in which SI = stimulation index; CPM_S = number of counts per minute of induced lymphocytes; CPM_C = number of counts per minute of non-induced lymphocytes.

2.5. Evaluation of IgE production

PBMCs were induced to IgE production for 12 days with 200 IU/ml rh-IL4 and 400 ng/ml rh-CD40L. On the 12th day, the culture supernatants were collected and IgE measurement was performed using human IgE enzyme-linked immunosorbent assay (ELISA) Kit according to the manufacturer's manual. Optical density (OD) of ELISA wells was absorption measured using an automated ELISA plate reader (Anthos, Austria) and the concentration of IgE was calculated according to standard curve and compared with the results of controls.

2.6. Statistical analysis

The data were analyzed using SPSS software version 16.0. Comparisons of categorical data between two groups (based on RT-PCR results) were performed using Fisher's exact test. Student's *T* test was used in all continuous numerical data including immunoglobulin levels, CD markers and switched memory B cell count (Aghamohammadi et al., 2011; Vodjgani et al., 2007); Odds ratio and 95% of confidence intervals was calculated for comparison of clinical phenotypes of patients based on previously described method (Aghamohammadi et al., 2010). Difference between groups was considered to be significant if the *P*-value was lower than 0.05.

3. Results

3.1. Characteristics of patients

Twenty-nine CVID patients (24 males and 5 females) were enrolled in this study. The mean (SD) age of the patients at the time of this study was 15.86 (9.42) years (median age: 14 years; ranged 4–51 years). The median age of the patient at the onset of disease was 24 months (ranged 1 month–15 years). The median age of the patients at the time of diagnosis was 72 months (ranged 4–43 years) and the median diagnostic delay was 42 months (ranged 1–336 months).

3.2. IgE mRNA expression

The results of RT-PCR on patients' samples showed that the 23 out of 29 patients (79.31%) were able to produce IgE mRNA comparable with normal controls while the remaining 6 patients (20.69%) were unable to do so. In contrast, IgE mRNA production was positive in all 21 controls (*P* = 0.033). Since the primer pairs were designed so that they specifically attach to the VH and Cε ends of the IgE antibody mRNA, the RT-PCR test becomes positive only after the occurrence of CSR and the production of IgE mRNA. As a result, subjects with positive RT-PCR should have normal CSR. As expected, the RT-PCR test showed normal mRNA production for *Actin* gene in all cases and controls which were used as an internal control.

3.3. B cell proliferation

From 6 CVID patients with impaired CSR, we were not able to test the B cell proliferation in 2 due to technical problems. In the 4 remaining cases, 2 patients (P7 and P20) had proliferation response comparable with normal controls while 2 other patients (P13 and P17) had reduced proliferation response compared to the controls. CPM_C and SI values of these selected patients are summarized in Table 2. Comparison of these 6 patients showed that they had lower level of IgG (76.4 ± 81.2 mg/dl vs. 235.4 ± 222.6 mg/dl; *P* = 0.04) and higher CD4/CD8 ratio (5.55 ± 0.8 vs. 0.89 ± 0.38 ; *P* < 0.001) significantly rather than remaining cases. Patients with impaired RT-PCR for IgE produced more IgE after stimulation than patients with normal result. There were no significant differences between clinical manifestation of these six cases and other CVID patients (Table 3).

3.4. IgE production

PBMCs of 16 out of 29 patients (55.2%) were not able to produce values of IgE comparable with those of the controls after being stimulated by IL-4 and CD40L. The mean (SD) of produced IgE was 4.35 (4.06) IU/ml in CVID patients and 14.49 (6.17) in controls (*P* < 0.001, Fig. 1). All 6 patients who had negative RT-PCR results for IgE mRNA were also unable to produce normal values of IgE after stimulation. The characteristics of patients, and the results of RT-PCR for *IgE* and *Actin* genes and IgE values before and after stimulation are shown in Table 2.

4. Discussion

Classification of CVID patients to more homogenous groups can improve their therapeutic management based on the type of an identified abnormality. The results of this study showed that 55.2% of CVID patients were unable to produce normal amounts of IgE protein after stimulation by IL-4 and CD40L compared to the controls. Moreover molecular investigations showed that 20.6% of all patients couldn't express IgE mRNA indicating impaired CSR in these patients. This difference between expression of IgE mRNA and IgE production in 34.6% of CVID cases may be interpreted by other defects rather than impaired CSR.

Table 2

Patients' characteristics, RT-PCR results for *IgE* and *Actin* genes, counts per minute (CPMs) and SI values of B cells' proliferation test and IgE values with and without stimulation by IL4 and CD40L.

ID	Sex	Age (years)	RT-PCR (<i>IgE</i>)	RT-PCR (<i>Actin</i>)	CPM _c	SI	Normal IgE (ng/ml)	Stimulated IgE (ng/ml)
P1	M	18	+	+			0.00	0.62*
P2	M	14	+	+			0.73	1.46*
P3	F	17	+	+			0.00	15.31
P4	M	13	+	+			0.24	1.22*
P5	M	21	+	+			0.00	1.46*
P6	M	10	+	+			0.73	1.22*
P7	F	7	–	+	9158.00	10.78	0.24	0.49*
P8	F	5	+	+			0.00	0.00*
P9	M	15	+	+			0.00	13.17
P10	M	14	+	+			0.00	0.00*
P11	M	21	+	+			0.82	.62*
P12	M	20	+	+			0.00	6.19
P13	M	24	–	+	3754.00*	6.21*	0.00	0.00*
P14	M	13	+	+			0.00	1.09*
P15	M	17	+	+			0.00	11.15
P16	M	29	+	+			0.00	10.85
P17	M	23	–	+	4634.00*	6.08*	0.00	0.00*
P18	M	27	–	+	ND	ND	0.00	0.00*
P19	M	51	+	+			0.12	6.34
P20	F	11	–	+	6792.00	10.13	0.00	0.00*
P21	M	8	+	+			0.00	1.65*
P22	M	15	–	+	ND	ND	0.00	0.00*
P23	M	7	+	+			0.00	5.23
P24	M	10	+	+			0.00	7.59
P25	M	13	+	+			0.00	6.32
P26	M	17	+	+			0.16	9.67
P27	M	4	+	+			0.00	4.31
P28	M	12	+	+			0.15	11.35
P29	M	15	+	+			0.11	8.95

M: male, F: female, *: defective compared with the controls, ND: not determined.

Subjects with normal CSR must have normal CD40–CD40L interactions and B lymphocytes' proliferation since stimulation of CD40L is essential in CSR signaling process. In contrast impaired CSR may be due to defects in stimulation of CD40L and assessment of B cells' proliferation can clarify the integrity of this signaling pathway.

Along with our study several investigations showed the abnormal Ig production in CVID patients in vitro (Clemente et al., 2011; Franz et al., 1992; Mayer et al., 1984; Pastorelli et al., 1990; Saxon et al., 1989). Pastorelli et al. (1990) reported that 6 out of 8 (75%) CVID patients couldn't produce measurable amounts of IgE following stimulation by anti-CD40 and IL-4. However, the expression of IgE mRNA was not investigated in this study. Franz et al. (1992) also observed that B cells of CVID patients stimulating by anti- μ and IL-2 could make normal amounts of IgM and IgG in 33.3% and low level of IgM but no IgG in 28.2%; while IgM and IgG were absent in 38.5% of cases.

In another study done by Mayer et al. (1984) only 11 out of 25 (44%) CVID patients could produce Ig plaque forming cell and only 45% and 9% of these responsive patients could also produce IgE secreting plaque forming cell and IgA secreting plaque forming cells respectively.

Nonoyama et al. (1993) assessed the function of B cells in 22 CVID patients with undetectable levels of IgE. In contrast to our results, the PBMCs of all patients could proliferate and synthesize normal amounts of IgE after stimulation by anti-CD40 and IL-4. Their results show that the signal receiving by B cells is appropriate. It has been explained that in CVID patients the T lymphocytes produce lower level of lymphokines with proper

stimulation in vitro (Blanco-Quiros et al., 2006; Nonoyama et al., 1993). Therefore, it seems that the antibody production defect observed in these patients is a failure in in vivo production of essential lymphokines for CSR.

In a complementary study, Eisenstein et al. (1994) showed defects in Ig production of CVID patients after stimulation with either anti-CD40 and IL-10 or *Staphylococcus aureus* Cowan I (SAC) and IL-2 and IL-10 that cannot be reversed by adding various cytokines such as Tumor growth factor beta (TGF- β). However they further reported normal expression of C μ , C γ , and C α mRNA after stimulation with anti-CD40 and IL-10 for 7 days and demonstrated a reversible defect in B cell differentiation in contrast to our study that the defect was irreversible, however, after stimulation. The difference could be possibly due to the smaller sample size of Eisenstein's study in comparison with ours.

The comparison of 6 patients with probable impaired CSR in our study with remaining cases showed significant reduction in level of B cells ($P = 0.001$). These patients also had decreased level of IgG and switched memory B cells; while their amount of CD4+ T cells was highly increased. As was demonstrated previously, binding of the CD40L to CD40 activates the proliferation and induces the CSR and leads to differentiation of B cells to specific Ig producing cells. This effect is amplified in the presence of cytokines (Armitage et al., 1993). The rate of B cells' proliferation in CVID patients can be a good indicator of their B response to CD40L.

According to all of these studies and our findings, we can classify CVID patients to three distinct groups: 1—patients

Table 3

Comparison of demographic, clinical and laboratory data of CVID patient based on the RT-PCR results for IgE gene.

Parameter	Impaired result (n = 6)	Normal result (n = 23)	P-value
Sex (male/female)	4/2	20/3	0.24
Parental consanguinity (yes/no)	4/2	14/9	0.47
Current age $\pm$ SD (year)	15.3 $\pm$ 9.8	17.8 $\pm$ 8.0	0.12
Onset age $\pm$ SD (year)	3.5 $\pm$ 0.4	3.2 $\pm$ 1.4	0.88
Diagnostic delay $\pm$ SD (year)	5.0 $\pm$ 0.6	4.8 $\pm$ 2.0	0.22
Infectious only phenotype	2	9	Reference OR
Poly lymphocytic infiltration phenotype	3	12	0.65
Autoimmunity phenotype	0	1	0.83
Malignancy phenotype	0	1	0.83
Enteropathy phenotype	1	0	0.25
IgE mRNA density (Δ copies/ μ g)	237.5 $\pm$ 108.7	210.0 $\pm$ 89.4	0.44
Total IgE (IU/ml)	5.4 $\pm$ 4.7	0.08 $\pm$ 0.01	<0.001*
IgG (mg/dl)	235.4 $\pm$ 222.6	76.4 $\pm$ 81.2	0.04*
IgA (mg/dl)	12.3 $\pm$ 1.5	5.7 $\pm$ 0.9	0.39
IgM (mg/dl)	36.0 $\pm$ 24.5	27.5 $\pm$ 17.1	0.34
White blood cell (cell/mm ³)	7640.4 $\pm$ 3307.4	7704 $\pm$ 1045.9	0.005*
Lymphocyte (cell/mm ³)	3352.8 $\pm$ 1430.7	3226.0 $\pm$ 1698.4	0.015*
T cell count (cell/mm ³)	2254.9 $\pm$ 1129.5	2530.8 $\pm$ 1356.1	0.002*
T helper (cell/mm ³)	1054.9 $\pm$ 1223.5	917.8 $\pm$ 490.0	0.029*
T cytotoxic count (cell/mm ³)	1398.0 $\pm$ 1959.7	1137.0 $\pm$ 543.1	0.002*
CD4/CD8	5.55 $\pm$ 0.8	0.89 $\pm$ 0.38	<0.001
B cell count (cell/mm ³)	387.8 $\pm$ 430.3	614.5 $\pm$ 552.6	0.001*
CD19+CD27+ switched memory B cell	0.22 $\pm$ 0.04	0.50 $\pm$ 0.07	0.08
IgE stimulated (IU/ml)	5.33 $\pm$ 4.85	0.04 $\pm$ 0.01	<0.001

with normal CSR and comparable amounts of IgE with healthy controls; 2—patients with normal CSR but undetectable IgE production; 3—patients with impaired CSR and consecutive impaired IgE production.

In the first group of patients, with no defect in IgE mRNA expression and protein production, the causative defect could be in other components of the innate and adaptive immunity such as dendritic cells and T lymphocytes.

In the second group of patients, with normal expression of IgE mRNA and impaired IgE production, investigations for processes occurring after mRNA production such as mRNA processing and transport, rapid degeneration of mRNA, and protein synthesis and secretion, etc. would help in the determination of the exact mechanism of defect. In the

remaining patients with defective CSR, investigation of molecular and cellular signaling pathways in CSR process should be considered.

In conclusion, CVID patients can be classified into more specific subgroups according to abnormalities in CSR process and the ability of consecutive IgE production. Determination of defects in CSR and its following processes such as IgE mRNA transcription, protein production, and secretion can help to clarify the various underlying mechanisms responsible for the development of CVID.

References

- Aghamohammadi, A., Farhoudi, A., Moin, M., Rezaei, N., Kouhi, A., Pourpak, Z., Yaseri, N., Movahedi, M., Gharagozlou, M., Zandieh, F., Yazadni, F., Arshi, S., Mohammadzadeh, I., Ghazi, B.M., Mahmoudi, M., Tahaei, S., Isaieian, A., 2005. Clinical and immunological features of 65 Iranian patients with common variable immunodeficiency. *Clin. Diagn. Lab. Immunol.* 12, 825.
- Aghamohammadi, A., Abolhassani, H., Moazzami, K., Parvaneh, N., Rezaei, N., 2010. Correlation between common variable immunodeficiency clinical phenotypes and parental consanguinity in children and adults. *J. Investig. Allergol. Clin. Immunol.* 20, 372.
- Aghamohammadi, A., Abolhassani, H., Biglari, M., Abolmaali, S., Moazzami, K., Tabatabaeiyan, M., Asgarian-Omran, H., Parvaneh, N., Mirahmadian, M., Rezaei, N., 2011. Analysis of switched memory B cells in patients with IgA deficiency. *Int. Arch. Allergy Immunol.* 156, 462.
- Armitage, R.J., Macduff, B.M., Spriggs, M.K., Fanslow, W.C., 1993. Human B cell proliferation and Ig secretion induced by recombinant CD40 ligand are modulated by soluble cytokines. *J. Immunol.* 150, 3671.
- Bayry, J., Hermine, O., Webster, D.A., Levy, Y., Kaveri, S.V., 2005. Common variable immunodeficiency: the immune system in chaos. *Trends Mol. Med.* 11, 370.
- Blanco-Quiros, A., Solis-Sanchez, P., Garrote-Adrados, J.A., Arranz-Sanz, E., 2006. Common variable immunodeficiency. Old questions are getting clearer. *Allergol. Immunopathol. (Madr)* 34, 263.
- Bonhomme, D., Hammarstrom, L., Webster, D., Chapel, H., Hermine, O., Le Deist, F., Lepage, E., Romeo, P.H., Levy, Y., 2000. Impaired antibody affinity maturation process characterizes a subset of patients with common variable immunodeficiency. *J. Immunol.* 165, 4725.

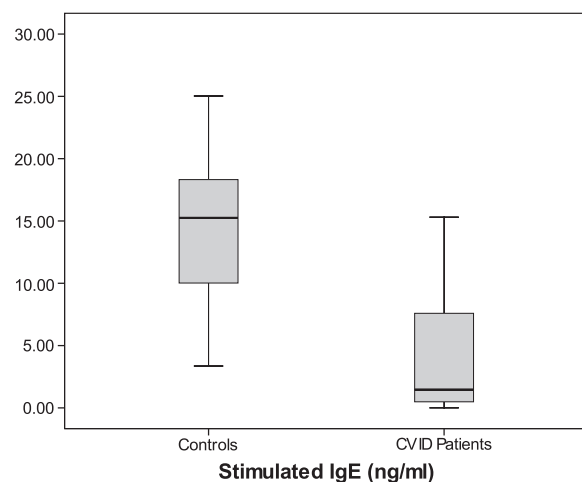


Fig. 1. Comparison of IgE production of PBMCs after stimulation by IL4 and CD40L in CVID patients and controls (P-value < 0.001).

- Castigli, E., Wilson, S.A., Garibyan, L., Rachid, R., Bonilla, F., Schneider, L., Geha, R.S., 2005. TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat. Genet.* 37, 829.
- Chapel, H.M., 1994. Consensus on diagnosis and management of primary antibody deficiencies. *BMJ* 308, 581.
- Clemente, A., Pons, J., Matamoros, N., Iglesias, J., Ferrer, J.M., 2011. 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. *Cell. Immunol.* 268, 9.
- Cunningham-Rundles, C., Bodian, C., 1999. Common variable immunodeficiency: clinical and immunological features of 248 patients. *Clin. Immunol.* 92, 34.
- Di Renzo, M., Pasqui, A.L., Auteri, A., 2004. Common variable immunodeficiency: a review. *Clin. Exp. Med.* 3, 211.
- Durandy, A., Taubenheim, N., Peron, S., Fischer, A., 2007. Pathophysiology of B-cell intrinsic immunoglobulin class switch recombination deficiencies. *Adv. Immunol.* 94, 275.
- Eisenstein, E.M., Chua, K., Strober, W., 1994. B cell differentiation defects in common variable immunodeficiency are ameliorated after stimulation with anti-CD40 antibody and IL-10. *J. Immunol.* 152, 5957.
- Franz, A., Bryant, A., Webster, A.D., Farrant, J., 1992. Effect of 12,13-phorbol dibutyrate and ionomycin on defective B cells in common variable immunodeficiency. *Clin. Exp. Immunol.* 87, 461.
- Kracker, S., Durandy, A., 2011. Insights into the B cell specific process of immunoglobulin class switch recombination. *Immunol. Lett.* 138, 97.
- Levy, Y., Gupta, N., Le Deist, F., Garcia, C., Fischer, A., Weill, J.C., Reynaud, C.A., 1998. Defect in IgV gene somatic hypermutation in common variable immuno-deficiency syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 95, 13135.
- Mayer, L., Fu, S.M., Cunningham-Rundles, C., Kunkel, H.G., 1984. Polyclonal immunoglobulin secretion in patients with common variable immuno-deficiency using monoclonal B cell differentiation factors. *J. Clin. Invest.* 74, 2115.
- Nonoyama, S., Farrington, M., Ishida, H., Howard, M., Ochs, H.D., 1993. Activated B cells from patients with common variable immunodeficiency proliferate and synthesize immunoglobulin. *J. Clin. Invest.* 92, 1282.
- Pastorelli, G., Roncarolo, M.G., Peronne, C., Tovo, P.A., de Vries, J.E., 1990. The capacity of interleukin-4 to induce in vitro IgE synthesis by B cells of patients with common variable immunodeficiency. *Clin. Exp. Immunol.* 82, 120.
- Salzer, U., Chapel, H.M., Webster, A.D., Pan-Hammarstrom, Q., Schmitt-Graeff, A., Schlesier, M., Peter, H.H., Rockstroh, J.K., Schneider, P., Schaffer, A.A., Hammarstrom, L., Grimbacher, B., 2005. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nat. Genet.* 37, 820.
- Saxon, A., Giorgi, J.V., Sherr, E.H., Kagan, J.M., 1989. Failure of B cells in common variable immunodeficiency to transit from proliferation to differentiation is associated with altered B cell surface-molecule display. *J. Allergy Clin. Immunol.* 84, 44.
- Sekine, H., Ferreira, R.C., Pan-Hammarstrom, Q., Graham, R.R., Ziemba, B., de Vries, S.S., Liu, J., Hippen, K., Koeuth, T., Ortmann, W., Iwahori, A., Elliott, M.K., Offer, S., Skon, C., Du, L., Novitzke, J., Lee, A.T., Zhao, N., Tompkins, J.D., Altshuler, D., Gregersen, P.K., Cunningham-Rundles, C., Harris, R.S., Her, C., Nelson, D.L., Hammarstrom, L., Gilkeson, G.S., Behrens, T.W., 2007. Role for Msh5 in the regulation of Ig class switch recombination. *Proc. Natl. Acad. Sci. U. S. A.* 104, 7193.
- Shapira, S.K., Vercelli, D., Jabara, H.H., Fu, S.M., Geha, R.S., 1992. Molecular analysis of the induction of immunoglobulin E synthesis in human B cells by interleukin 4 and engagement of CD40 antigen. *J. Exp. Med.* 175, 289.
- van Zelm, M.C., Reisli, I., van der Burg, M., Castano, D., van Noesel, C.J., van Tol, M.J., Woellner, C., Grimbacher, B., Patino, P.J., van Dongen, J.J., Franco, J.L., 2006. An antibody-deficiency syndrome due to mutations in the CD19 gene. *N. Engl. J. Med.* 354, 1901.
- Vodjani, M., Aghamohammadi, A., Samadi, M., Moin, M., Hadjati, J., Mirahmadian, M., Parvaneh, N., Salavati, A., Abdollahzade, S., Rezaei, N., Srrafnejad, A., 2007. Analysis of class-switched memory B cells in patients with common variable immunodeficiency and its clinical implications. *J. Investig. Allergol. Clin. Immunol.* 17, 321.