Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2006.03274.x

T and B lymphocyte subpopulations and activation/differentiation markers in patients with selective IgA deficiency

J. Litzman, M. Vlková, Z. Pikulová, D. Štikarovská and J. Lokaj
Department of Clinical Immunology and Allergology, St Anne's University Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Summary

Selective deficiency of immunoglobulin A (IgAD) and common variable immunodeficiency (CVID) are genetically closely related diseases, both of unknown pathogenesis. A plethora of abnormalities in lymphocyte subpopulations and expression of activation markers were repeatedly documented in CVID patients, while almost no data are available about lymphocyte subpopulations in IgAD patients. We determined basic lymphocyte subpopulations and those subpopulations that were reported to be abnormal in CVID patients (CD25, human leucocyte antigen (HLA)-DR CD45RA, CD45RO, CD27, CD28 and CD29 on both $CD4^+$ and $CD8^+$ cells, CD57 and CD38 on $CD8^+$ cells, CD21, CD27, IgM, IgD on B lymphocytes) in 85 patients with IgAD, 47 patients with CVID and in 65 healthy controls. Statistical analysis was performed by the Mann–Whitney *U*-test; significant *P*-values were determined by means of Bonferoni's correction. Our results showed an increase in the relative number of $CD8^+$ cells and a decrease in the absolute number of $CD4^+$ cells compared to healthy people, but similar abnormalities in CVID patients were much more expressed. IgAD patients had significantly decreased expression of HLA-DR and increased expression of CD25 on $CD4^+$ lymphocytes, also CD29 expression was decreased on $CD8^+$ cells, while other activation/differentiation markers on T cells (including the expression of CD45RA and CD45RO antigens) were not changed. There were no statistically significant abnormalities in B lymphocyte developmental stages in IgAD patients compared to healthy controls. Our observation showed that the majority of T and B lymphocyte subpopulation abnormalities described previously in CVID are not present in IgAD patients.

Keywords: common variable immunodeficiency, B lymphocyte subsets, differentiation antigens, IgA deficiency, T lymphocyte subsets

Accepted for publication 31 October 2006
Correspondence: Dr J. Litzman, Department of Clinical Immunology and Allergology, St Anne's University Hospital, Faculty of Medicine, Masaryk University, Brno, Czech Republic.
E-mail: jiri.litzman@fnusa.cz

Introduction

Selective deficiency of immunoglobulin A (IgAD) is the most frequent, but usually asymptomatic, abnormality of immunoglobulin production, with a frequency ranging from 1 : 278 to 1 : 3040 in Caucasians [1]. Despite its high frequency, very little is known about the aetiopathogenic mechanisms leading to this immunological abnormality.

IgAD is linked to a more severe immunoglobulin production disease – common variable immunodeficiency (CVID), which is characterized by decreased IgG and IgA levels, while IgM levels are variable. Clinically significant immunodeficiency evolves at any age after apparently normal function of the immune system [2]. Familial occurrence of both diseases has been documented repeatedly [3,4]. Deficiency of transmembrane activator and CALM interactor (TACI) was observed both in CVID and IgAD patients [5,6]. All these results show that IgAD and CVID may be different phenotypic manifestations of the same, or similar, genetic background. Repeatedly documented progression of IgAD into CVID [7] shows that IgAD may be a developmental stage in evolving CVID.

Although the pathogenesis of CVID has not yet been elucidated, many abnormalities in the number or function of immune cells were described in CVID patients. Great attention has been given to abnormalities in lymphocyte subpopulations. Defective activation of T lymphocytes

[8–10] and impaired cytokine production [11–13] were observed in CVID patients. Various T subpopulation abnormalities including a decreased number of $CD4^+$ cells [14–16], reduced expression of CD45RA [15] and CD62L [17] or increased expression of CD45RO [15] on $CD4^+$ cells, human leucocyte antigen (HLA)-DR [16] and CD57 on $CD8^+$ [18] were reported. Also, decreased expression of CD27, CD28, CD29 on both $CD4^+$ and $CD8^+$ cells and increased expression of CD38 and CD57 on $CD8^+$ cells were observed [19]. When evaluating B lymphocyte subpopulations, a decrease in the number of memory $CD27^+$ IgD^- IgM B cells has been described repeatedly [20–24].

Despite the assumed relation of CVID and IgAD, there are almost no data available about T and B lymphocyte subpopulations in IgAD. Previous studies performed on limited numbers of patients showed a possible decrease of the proportion of $CD4^+$ cells and increase of proportion of $CD8^+$ cells [25–27], while Klemola *et al.* [28] did not observe any significant abnormalities in relative numbers of $CD3^+$, $CD4^+$ and $CD8^+$ cells. Because of the above-mentioned similar genetic background of CVID and IgAD, assessing those abnormalities in IgAD may help to elucidate whether lymphocyte abnormalities in CVID are primary, inherited or whether they evolve secondarily during the development of the diseases, or are even a consequence of immunodeficiency in CVID.

In our study we have evaluated lymphocyte subpopulations most frequently known to be abnormal in CVID patients in a group of 85 patients with IgAD, 65 controls and 47 patients with CVID.

Patients and methods

Eighty-five patients with IgAD fulfilling diagnostic criteria of IgAD [29] (48 females, 37 males) aged 18–72 years were included in the study. These patients were referred most frequently for immunological investigation because of apparently increased frequency of mild respiratory tract infections; none of them suffered from clinically significant immunodeficiency or clinically apparent autoimmune disease. Lymphocyte subpopulations were also determined in 47 patients with CVID (30 females, 17 males) aged 10–75 years. A detailed analysis of relations of lymphocyte subpopulations in the CVID subgroup has been published previously [19]. As a control group, 65 healthy people with normal serum IgA levels (35 females, 30 males) aged 22–66 years were used. Controls were recruited from hospital staff and medical students. All blood samples were obtained in an acute infection-free period; in CVID patients on IVIG treatment, blood samples were obtained prior to immunoglobulin infusion.

The study was approved by the Ethics Committee of the Faculty of Medicine of Masaryk University in Brno. Informed consent was obtained from all investigated individuals before the samples were drawn.

Lymphocyte subpopulation determination

All blood samples were taken between 8 and 11 a.m. to exclude diurnal variation of lymphocyte subsets. B lymphocyte subpopulations were analysed from density gradient separated cells while T lymphocyte subpopulations were determined from non-separated blood, as described previously [19]. Immunophenotyping of lymphocytes was performed by four-colour cytometry Epics MCL (Beckman Coulter Miami, FL, USA).

Major lymphocyte populations were determined by two cocktails of monoclonal antibodies (MoAb): 1, anti-CD45 fluorescein isothiocyanate (FITC), anti-CD4 phycoerythrin (PE), anti-CD8 phycoerythrin-Texas red X (ECD) and anti-CD3PC5; 2, anti-CD45FITC, anti-CD56PE, anti-CD19ECD, anti-CD3 r-phycoerythrin-cyanine 5 (PC5); (Cyto-Stat tetraCHROME; Beckman Coulter, Miami, FL, USA) and anti-CD16PE (Immunotech, Marseille, France). In addition, the following MoAbs were used: PE-conjugated anti-CD38, CD45RA, CD45RO, FITC-conjugated anti-CD25, CD27, CD28, CD29, CD57, CD62L, PC5-conjugated anti-CD3 (all from Dako A/S, Glostrup, Denmark); PC5-conjugated anti-CD27, ECD-conjugated anti-CD4, CD8, CD19 (all from Beckman Coulter); FITC-conjugated anti-CD38, PE-conjugated anti-IgD and anti-CD21, PC5-conjugated anti-IgM (all from Pharmingen International, San Diego, CA, USA) and PE-conjugated anti-HLA-DR (Becton Dickinson, San Jose, CA, USA).

Statistical analysis

Normality of the obtained lymphocyte subpopulations was assessed by the Kolmogorov–Smirnov test. Because the majority of the obtained values showed a distribution different from normal, a non-parametric Mann–Whitney *U*-test was used. Critical values for statistical significance were determined by Bonferroni's correction [30] for each group of lymphocytes: absolute and relative numbers of major lymphocyte subpopulations, $CD4^+$ activation/differentiation markers, $CD8^+$ activation/differentiation markers, absolute and relative numbers of B cell activation states. Statistical package STATISTICA (StatSoft, Inc.) version 7 was used.

Results

The absolute and relative numbers of $CD3^+$, $CD4^+$ and $CD8^+$ T lymphocytes in IgAD patients, healthy controls and CVID patients are shown in Tables 1 and 2. As can be seen, the absolute number of $CD4^+$ cells was decreased and the relative number of $CD8^+$ cells was increased in IgAD patients compared to healthy controls. Comparing CVID patients with IgAD patients, the relative numbers of $CD3^+$, $CD8^+$ cells were increased in CVID patients while $CD4^+$ and natural killer (NK) ($CD16/56^+$ $CD3^-$) cells were decreased compared to IgAD patients. Evaluating absolute numbers, the number of

Table 1. Relative numbers of basic lymphocyte subpopulations in IgAD, CVID and controls. Results are expressed as median ± standard deviation (s.d.). Lymphocytes are expressed as percentage of $CD45^+$ cells, lymphocyte subpopulations as percentage of lymphocytes.

	Controls ($n = 65$)	IgAD ($n = 85$)	CVID ($n = 47$)	*P*, IgAD controls	*P*, IgAD CVID
Lymphocytes (%)	31·0 ± 7·8	29·0 ± 7·5	24·0 ± 10·7	0·084	0·007*
$CD3^+$ (%)	74·0 ± 7·5	73·0 ± 7·1	79·0 ± 9·1	0·799	< 0·001*
$CD4^+$ (%)	45·9 ± 8·5	42·7 ± 8·2	37·8 ± 9·5	0·015	< 0·001*
$CD8^+$ (%)	21·8 ± 7·4	25·9 ± 5·8	36·0 ± 11·4	< 0·001*	< 0·001*
$CD19^+$ (%)	11·09 ± 3·1	12·0 ± 4·0	10·0 ± 7·3	0·094	0·099
$CD16/56^+$ $CD3^-$ (%)	15·0 ± 7·8	13·0 ± 6·4	8·0 ± 7·1	0·422	0·004*

*Indicates statistically significant difference using critical value after Bonferroni's correction: 0·008. CVID: common variable immunodeficiency; IgAD: selective deficiency of immunoglobulin A.

CD4 cells and NK cells was also decreased significantly in CVID patients compared to IgAD patients.

Assessing the expression of activation/differentiation markers on $CD4^+$ cells, a decreased expression of HLA-DR on $CD4^+$ cells and an increased expression of CD25 on $CD4^+$ cells was observed in IgAD patients compared to controls; this increase was also significant when comparing IgAD patients with CVID patients. No other significant differences were observed when comparing IgAD and controls. When comparing IgAD patients with CVID patients, an increase in the expression of CD45RA and a decrease in the expression of CD45RO and HLA-DR was observed in IgAD patients. Also, the number of $CD28^+$ cells was increased and that of $CD29^+$ cells decreased in IgAD compared to CVID patients (see Table 3).

When assessing activation/differentiation markers on $CD8^+$ cells, only a decrease in $CD29^+$ expression was observed comparing IgAD and healthy people or CVID patients, while almost in all parameters significant differences were observed between CVID and IgAD patients (decrease in CD45RO, CD57, CD38, HLA-DR and increase in CD25, CD27 and CD28 when comparing IgAD to CVID patients) (see Table 4).

There were no significant abnormalities in the absolute and relative numbers of B lymphocyte differentiation stages when comparing IgAD and controls, while in CVID patients typical abnormalities described previously by others [20–24]

Table 2. Absolute numbers of basic lymphocyte subpopulations in IgAD, CVID and controls. Results are expressed as median ± standard deviation (s.d.).

	Controls ($n = 65$)	IgAD ($n = 85$)	CVID ($n = 47$)	*P*, IgAD controls	*P*, IgAD CVID
Leucocytes (10^9/l)	6·1 ± 1·5	6·1 ± 1·6	6·3 ± 2·3	0·962	0·483
Lymphocytes (10^9/l)	1·89 ± 0·12	1·77 ± 0·12	1·61 ± 0·25	0·170	0·186
$CD3^+$ (10^9/l)	1·32 ± 0·49	1·25 ± 0·43	1·22 ± 0·77	0·231	0·483
$CD4^+$ (10^9/l)	0·89 ± 0·36	0·75 ± 0·28	0·54 ± 0·32	0·006*	< 0·001*
$CD8^+$ (10^9/l)	0·39 ± 0·32	0·43 ± 0·20	0·58 ± 0·50	0·143	0·023
$CD19^+$ (10^9/l)	0·21 ± 0·09	0·20 ± 0·11	0·15 ± 0·18	0·905	0·023
$CD16/56^+$ $CD3^-$ (10^9/l)	0·24 ± 9·20	0·22 ± 0·13	0·14 ± 0·11	0·117	0·003*

*Indicates statistically significant difference using critical value after Bonferroni's correction: 0·007. CVID: common variable immunodeficiency; IgAD: selective deficiency of immunoglobulin A.

Table 3. Activation/differentiation markers on $CD4^+$ cells on IgAD, CVID and controls. Results are expressed as median ± standard deviation (s.d.).

	Controls ($n = 65$)	IgAD ($n = 85$)	CVID ($n = 47$)	*P*, IgAD controls	*P*, IgAD CVID
$CD4^+$ $CD45RA^+/CD4^+$ (%)	44·9 ± 16·2	45·0 ± 14·4	24·3 ± 16·3	0·775	< 0·001*
$CD4^+$ $CD45RO^+/CD4^+$ (%)	49·7 ± 15·1	54·1 ± 15·1	75·4 ± 18·9	0·597	< 0·001*
$CD4^+$ $HLA\text{-}DR^+/CD4^+$ (%)	6·9 ± 6·4	3·9 ± 3·6	10·2 ± 8·2	< 0·001*	< 0·001*
$CD4^+$ $CD25^+/CD4^+$ (%)	3·5 ± 3·1	9·7 ± 4·0	6·5 ± 7·9	< 0·001*	< 0·001*
$CD62L^+$ $CD4^+/CD4^+$ (%)	75·3 ± 11·8	74·6 ± 11·2	71·9 ± 18·4	0·887	0·131
$CD4^+$ $CD27^+/CD4^+$ (%)	89·0 ± 8·5	86·3 ± 7·9	85·9 ± 12·8	0·039	0·314
$CD4^+$ $CD28^+/CD4^+$ (%)	98·3 ± 4·6	98·3 ± 3·6	95·6 ± 5·9	0·868	< 0·001*
$CD4^+$ $CD29^+/CD4^+$ (%)	50·4 ± 12·5	47·2 ± 13·4	65·3 ± 16·6	0·197	< 0·001*

*Indicates statistically significant difference using critical value after Bonferroni's correction: 0·006. CVID: common variable immunodeficiency; IgAD: selective deficiency of immunoglobulin A.

Table 4. Activation/differentiation markers on $CD8^+$ cells of IgAD, CVID and control. Results are expressed as median ± standard deviation (s.d.).

	Controls ($n = 65$)	IgAD ($n = 85$)	CVID ($n = 47$)	*P*, IgAD controls	*P*, IgAD CVID
$CD8^+$ $CD45RA^+/CD8^+$ (%)	62·4 ± 13·5	58·5 ± 14·8	54·8 ± 16·2	0·197	0·131
$CD8^+$ $CD45RO^+/CD8^+$ (%)	34·5 ± 13·6	28·9 ± 13·1	41·2 ± 14·2	0·010	< 0·001*
$CD8^+$ $CD57/CD8^+$ (%)	17·3 ± 15·0	18·5 ± 12·9	34·7 ± 14·7	0·965	< 0·001*
$CD8^+$ $CD38/CD8^+$ (%)	13·3 ± 8·3	10·0 ± 7·1	23·8 ± 17·0	0·014	0·001*
$CD8^+$ $CD25^+/CD8^+$ (%)	1·1 ± 3·2	1·7 ± 5·6	0·6 ± 5·4	0·346	< 0·001*
$CD8^+$ $HLA\text{-}DR^+/CD8^+$ (%)	6·2 ± 6·2	2·4 ± 4·6	14·5 ± 15·8	0·280	0·005*
$CD62L^+$ $CD8^+/CD8^+$ (%)	40·3 ± 17·5	39·2 ± 15·5	34·5 ± 19·1	0·603	0·151
$CD8^+$ $CD27^+/CD8^+$ (%)	69·2 ± 19·2	58·0 ± 15·6	42·5 ± 22·4	0·022	0·001*
$CD8^+$ $CD28^+/CD8^+$ (%)	65·7 ± 20·4	58·8 ± 17·9	35·6 ± 20·4	0·030	0·002*
$CD8^+$ $CD29^+/CD8^+$ (%)	93·4 ± 7·4	79·1 ± 12·8	92·4 ± 21·7	< 0·001*	< 0·001*

*Indicates statistically significant difference using critical value after Bonferroni's correction: 0·005. CVID: common variable immunodeficiency; IgAD: selective deficiency of immunoglobulin A.

were observed when compared to IgAD patients (Table 5). When assessing B lymphocyte numbers according to the Freiburg [22] classification of CVID, no major abnormalities were observed. Only one IgAD patients and one control person fell into group Ib.

Discussion

The goal of our study was to determine whether lymphocyte subpopulation abnormalities observed in CVID patients were also present in a genetically related disorder – IgAD. The results showed that the longest known abnormalities in CVID – the decrease in $CD4^+$ cells and the increase in the relative number of $CD8^+$ cells – can also be documented in IgAD patients although, in our results, these abnormalities were demonstrated only in absolute numbers of $CD4^+$ cells and in relative numbers of $CD8^+$ cells. The decrease in $CD4^+$ cells was ascribed to the decrease in $CD45RA^+$ cells [15], but we could not detect this abnormality in our IgAD patients; the percentage of CD45RA and also CD45RO was normal both in $CD4^+$ and $CD8^+$ cells.

A surprising observation was that of a decrease in the expression of HLA-DR on $CD4^+$ cells of IgAD patients compared to both CVID patients and healthy controls, and an increase in the CD25 expression on $CD4^+$ cells, again compared to both CVID and IgAD patients. Razziudin *et al.* [25] also described increased expression of Tac (CD25) antigen on peripheral blood lymphocytes in two of three IgAD patients; these individuals had an increased proportion of OKDR (HLA-DR) and OKIa1 (HLA-DR)-positive peripheral lymphocytes. HLA-DR and CD25 are both activation markers of T lymphocytes; CD25 is an earlier activation marker compared to HLA-DR [31]. We cannot explain the cause of possible activation of $CD4^+$ cells in our IgAD patients because they were investigated in an infection-free period and none of them had clinically apparent autoimmune disease at the time of investigation. Surprisingly, no other activation abnormalities were observed in IgAD patients compared to controls. As well as on the activated lymphocytes, CD25 is also expressed on T regulated (T_{reg}) cells; we cannot exclude the possibility that this subpopulation abnormality influences the above-mentioned difference

Table 5. Absolute and relative numbers of differentiation stages of B cells. CVID patients with B lymphocytes < 1% were excluded. Naive B lymphocytes were defined as: $CD19^+$ $CD27^-$ IgM^+ IgD^+ cells, IgM memory as: $CD19^+$ $CD27^+$ IgM^+ IgD^+ cells, switched memory as: $CD19^+$ $CD27^+$ IgM^- IgD^- cells, immature as $CD19^+$ IgM^+ $CD21^-$.

	Controls ($n = 65$)	IgAD ($n = 85$)	CVID ($n = 40$)	*P*, IgAD controls	*P*, IgAD CVID
Naive (10^9/l)	0·099 ± 0·053	0·111 ± 0·072	0·142 ± 0·139	0·308	0·271
IgM memory (10^9/l)	0·046 ± 0·025	0·043 ± 0·031	0·015 ± 0·043	0·902	< 0·001*
Switched memory (10^9/l)	0·034 ± 0·038	0·028 ± 0·024	0·004 ± 0·012	0·108	< 0·001*
IgM^+ $CD21^-$ (10^9/l)	0·009 ± 0·011	0·012 ± 0·015	0·029 ± 0·064	0·034	< 0·001*
Naive/B cells (%)	52.8 ± 12·5	56·8 ± 14·5	83·3 ± 15·8	0·135	< 0·001*
Naive/PBL (%)	1·8 ± 1·1	1·6 ± 1·1	0·2 ± 0·6	0·203	< 0·001*
IgM memory/B cells (%)	22·6 ± 7·4	20·5 ± 10·4	8·0 ± 12·3	0·520	< 0·001*
Switched memory/B cells (%)	19·6 ± 7·5	14·9 ± 7·5	1·8 ± 3·8	0·039	< 0·001*
IgM^+ $CD21^-$/B cells (%)	4·8 ± 4·3	6·7 ± 4·2	14·8 ± 14·8	0·025	< 0·001*

*Indicates statistically significant difference using critical value after Bonferroni's correction: 0·006. CVID: common variable immunodeficiency; IgAD: selective deficiency of immunoglobulin A.

between IgAD and controls and also CVID patients. Unfortunately, there are no data available about the number of T_{reg} cells in IgAD patients. Similar changes as in $CD4^+$ cells were observed in $CD8^+$ lymphocytes, although they were less intensive, not reaching statistical significance when comparing IgAD and controls.

Unlike in CVID, we could not document any statistically significant abnormalities in the differentiation stages of B cells, only a tendency to a decreased number of switched memory cells and a mild increase in IgM^+ $CD21^-$ immature cells was present, but these abnormalities were much less extensive than those observed in CVID patients. In IgAD patients, a reduced but still significant number of IgA^+ B cells can be documented [28]. On a molecular level, an Iα germline transcript expression was reduced and α circle transcripts were not detected in three IgAD patients [32]. Another study showed normal Iα germline transcription in four IgAD patients, but in two of them the Sμ–Sα switch recombination was impaired while in the remaining two decreased secreted and membrane Cα mRNA was markedly decreased in IgA switched B cells [33]. All these results show a probable heterogeneity in the B cell defect of IgAD patients, but according to our results, unlike the situation in CVID, such abnormalities do not affect maturation of the majority of B cells, as reflected by lymphocyte activation marker determination in blood.

In conclusion, our study showed that, with the exception of abnormalities in the numbers of $CD4^+$ and $CD8^+$ cells, no major abnormalities of lymphocyte subpopulations or expression of activation/differentiation markers were observed in IgAD patients. These results may show that lymphocyte subpopulation abnormalities seen in CVID are probably not a consequence of an inborn genetic defect but that they evolve as a part, or as a consequence, of immunodeficiency in CVID.

Acknowledgements

This work was supported by grant no. NI/7981–3 from the Czech Ministry of Health.

References

1 Weber-Mzell D, Kotanko P, Hauer AC *et al.* Gender, age and seasonal effects on IgA deficiency: a study of 7293 Caucasians. Eur J Clin Invest 2004; **34**:224–8.

2 Hammarström L, Vorechovsky I, Webster D. Selective IgA deficiency (SIgAD) and common variable immunodeficiency (CVID). Clin Exp Immunol 2000; **120**:225–31.

3 Vorechovsky I, Zetterquist H, Paganelli R *et al.* Family and linkage study of selective IgA deficiency and common variable immunodeficiency. Clin Immunol Immunopathol 1995; **77**:185–92.

4 Litzman J, Sevcikova I, Stikarovska D, Pikulova Z, Pazdirkova A, Lokaj J. IgA deficiency in Czech healthy individuals and selected patient groups. Int Arch Allergy Immunol 2000; **123**:177–80.

5 Castigli E, Wilson SA, Garibyan L *et al.* TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet 2005; **37**:829–34.

6 Salzer U, Chapel HM, Webster AD *et al.* Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. Nat Genet 2005; **37**:820–8.

7 Espanol T, Catala M, Hernandez M, Caragol I, Bertran JM. Development of a common variable immunodeficiency in IgA-deficient patients. Clin Immunol Immunopathol 1996; **80**:333–5.

8 Fischer MB, Hauber I, Eggenbauer H *et al.* A defect in the early phase of T-cell receptor-mediated T-cell activation in patients with common variable immunodeficiency. Blood 1994; **84**:4234–41.

9 Thon V, Wolf HM, Sasgary M *et al.* Defective integration of activating signals derived from the T cell receptor (TCR) and costimulatory molecules in both CD4+ and CD8+ T lymphocytes of common variable immunodeficiency (CVID) patients. Clin Exp Immunol 1997; **10**:174–81.

10 Fischer MB, Wolf HM, Hauber I *et al.* Activation via the antigen receptor is impaired in T cells, but not in B cells from patients with common variable immunodeficiency. Eur J Immunol 1996; **26**:231–7.

11 Holm AM, Aukrust P, Aandahl EM, Muller F, Tasken K, Fröland SS. Impaired secretion of IL-10 by T cells from patients with common variable immunodeficiency–involvement of protein kinase A type I. J Immunol 2003; **170**:5772–7.

12 North ME, Webster AD, Farrant J. Primary defect in CD8+ lymphocytes in the antibody deficiency disease (common variable immunodeficiency): abnormalities in intracellular production of interferon-gamma (IFN-gamma) in CD28+ ('cytotoxic') and CD28-('suppressor') CD8+ subsets. Clin Exp Immunol 1998; **111**:70–5.

13 Ferrer JM, Iglesias J, Hernandez M, Matamoros N. Alterations in interleukin secretion (IL-2 and IL-4) by CD4 and CD4 CD45RO cells from common variable immunodeficiency (CVI) patients. Clin Exp Immunol 1995; **102**:286–9.

14 Farrant J, Spickett G, Matamoros N *et al.* Study of B and T cell phenotypes in blood from patients with common variable immunodeficiency (CVID). Immunodeficiency 1994; **5**:159–69.

15 Lebranchu Y, Thibault G, Degenne D, Bardos P. Abnormalities in CD4+ T lymphocyte subsets in patients with common variable immunodeficiency. Clin Immunol Immunopathol 1991; **61**:83–92.

16 Guazzi V, Aiuti F, Mezzaroma I *et al.* Assessment of thymic output in common variable immunodeficiency patients by evaluation of T cell receptor excision circles. Clin Exp Immunol 2002; **129**:346–53.

17 Nordoy I, Müller F, Aukrust P, Froland SS. Adhesion molecules in common variable immunodeficiency (CVID) – a decrease in 1-selectin-positive T lymphocytes. Clin Exp Immunol 1998; **114**:258–63.

18 Baumert E, Wolff-Vorbeck G, Schlesier M, Peter HH. Immunophenotypical alterations in a subset of patients with common variable immunodeficiency (CVID). Clin Exp Immunol 1992; **90**:25–30.

19 Vlkova M, Thon V, Sarfyova M *et al.* Age dependency and mutual relations in T and B lymphocyte abnormalities in common variable immunodeficiency patients. Clin Exp Immunol 2006; **143**:373–9.

20 Brouet JC, Chedeville A, Fermand JP, Royer B. Study of the B cell memory compartment in common variable immunodeficiency. Eur J Immunol 2000; **30**:2516–20.

21 Agematsu K, Futatani T, Hokibara S *et al.* Absence of memory B cells in patients with common variable immunodeficiency. Clin Immunol 2002; **103**:34–42.
22 Warnatz K, Denz A, Dräger R *et al.* Severe deficiency of switched memory B cells ($CD27^{+}IgM^{-}IgD^{-}$) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood 2002; **99**:1544–51.
23 Ferry BL, Jones EA, Bateman EA *et al.* Measurement of peripheral B cell subpopulations in common variable immunodeficiency (CVID) using a whole blood method. Clin Exp Immunol 2005; **140**:532–9.
24 Piqueras B, Lavenu-Bombled C, Galicier L *et al.* Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol 2003; **23**:385–400.
25 Raziuddin S, Elawad ME, Benjamin B. T-cell abnormalities in antibody deficiency syndromes. Scand J Immunol 1989; **30**:419–24.
26 Litzman J, Stouracova M, Krejci M, Thon V, Lokaj J. T-lymphocytes in IgA deficiency. Scripta Med Facult Med Univ Brunensis Masarykianae 2001; **74**:345–52.
27 Melamed I, Zakuth V, Kark JO, Spirer Z. The immune system in isolated IgA deficiency. J Clin Lab Immunol 1985; **17**:163–6.
28 Klemola TK, Eskola J, Savilahti E. T- and B-cell functions in IgA-deficient patients. Scand J Immunol 1988; **28**:301–6.
29 Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Clin Immunol 1999; **93**:190–7.
30 Winer BJ, Brown DR, Michels KM. Statistical principles in experimental design, 3rd edn. New York: McGraw-Hill, 1991.
31 Caruso AS, Licenziati M, Corulli AD *et al.* Flow cytometric analysis of activation markers on stimulated T cells and their correlation with cell proliferation. Cytometry 1997; **27**:71–6.
32 Asano T, Kaneko H, Terada T *et al.* Molecular analysis of B-cell differentiation in selective or partial IgA deficiency. Clin Exp Immunol 2004; **136**:284–90.
33 Wang Z, Yunis D, Irigoyen M *et al.* Discordance between IgA switching at the DNA level and IgA expression at the mRNA level in IgA-deficient patients. Clin Immunol 1999; **91**:263–70.