

Terminally differentiated memory T cells are increased in patients with common variable immunodeficiency and selective IgA deficiency

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Abstract

Introduction: Previous studies showed that several lymphocyte abnormalities seen in the most frequent symptomatic immunoglobulin deficiency, common variable immunodeficiency (CVID), were also observed in a genetically related asymptomatic disorder – selective IgA deficiency (IgAD). In this study we searched for abnormalities in the differentiation stages of T cells as well as for similarities of these abnormalities in CVID and IgAD patients.

Material and methods: Using flow cytometry in 80 patients with IgAD, 48 patients with CVID, and 80 control persons we determined T-lymphocyte subsets: both CD4 and CD8 were divided into the naïve CD45RO⁻CD27⁺, early differentiated CD45RO⁺CD27⁺, late differentiated CD45RO⁺CD27⁻ and fully differentiated effector CD45RO⁻CD27⁻ memory T cells, as well as Treg cells, defined as CD4⁺C-D25^{high}CD127^{low} T cells.

Results: An increase of CD4⁺ and CD8⁺ late differentiated memory cells was observed comparing CVID patients to controls, as well as comparing IgAD patients to controls. In CVID patients an increase of CD4⁺ early differentiated memory cells, a decrease of CD8⁺ intermediate memory cells, and CD4⁺ and CD8⁺ naïve cells were found as well. The abnormalities in IgAD patients might be explained by higher CMV seropositivity observed in our IgAD. We confirmed the repeatedly published decrease of Treg cells in CVID patients, while Treg cells in IgAD patients were increased compared to controls.

Conclusions: Our results show T-cell activation not only in CVID, but also in IgAD patients. The increase in IgAD patients may be influenced by a more frequent CMV infection in our group of IgAD patients.

Key words: IgA deficiency, common variable immunodeficiency, T-lymphocyte subpopulations, CMV.

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Introduction

The pathogenesis of selective IgA deficiency (IgAD, serum IgA < 0.07 g/l, and normal other immunoglobulin isotype levels), the most frequent immunoglobulin deficiency in Caucasians, is unknown [1]. Patients with IgAD are asymptomatic in most cases, or they may suffer from recurrent infections of the respiratory tract; also the frequency of autoimmune diseases is increased in IgAD [2]. Compared to this, in the most frequent symptomatic hypogammaglobulinaemia: common variable immunodeficiency (CVID), besides IgA, also IgG and frequently IgM serum levels are decreased [3]. The clinical manifestation of CVID is diverse,

the patients suffer from recurrent and complicated infections mainly of the respiratory tract, but also from autoimmune diseases, splenomegaly, gastrointestinal diseases, or systemic granulomatous disease [4-6]. CVID seems to be a group of different diseases with similar clinical and laboratory manifestation, and we cannot exclude a similar situation in IgAD. However, the occurrence of both diseases in affected families and the repeatedly described progression of IgAD to CVID suggest a similar genetic background of IgAD and CVID, at least in some cases [7, 8].

Various abnormalities in the number or function of T and B cells were described in patients with CVID [9-15].

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Assessing T cells, a decrease in CD4⁺ and an increase in CD8⁺ T cells were repeatedly described, also an increase in memory CD4⁺CD45RO⁺ [9, 11, 12], activated CD4⁺CD25⁺ [11, 12], CD8⁺HLA-DR⁺ [9, 11-13], CD8⁺CD38⁺ [11, 12], terminally differentiated CD8⁺CD57⁺ [9, 12, 13], or a decrease in, naïve CD4⁺CD45RA⁺CD27⁻ [11, 12], and non-effector CD4⁺CD27⁺ [11], CD8⁺CD27⁺ [11, 12], or regulatory (T_{reg}) T cells [14, 16] were reported. Although with a markedly less significant expression, some of these abnormalities in T [11, 12] and B [10-12] cells were described in IgAD as well, showing that the immunopathogenesis of both diseases may be similar. The elevated numbers of activated CD4⁺ and CD8⁺ lymphocytes, but also higher serum neopterin [17] or soluble CD25 (sCD25) levels [18], point to the activation of the immune system in patients with CVID and possibly IgAD.

The development and maturation of T cells in periphery is a complex process, and various schemes are used to describe the differentiation stages of T lymphocytes in peripheral blood [19-21]. In our study, we intended to determine the T-cell differentiation stages spectrum in CVID and IgAD patients. For the differentiation stages of T cells we used the scheme described by Maenetje [22] based on the determination of CD45RO and CD27. Because our previous studies showed that many abnormalities in B-cell subsets seen in CVID were also observed in IgAD patients [10-12], we have also searched for similarities and dissimilarities in T-cell differentiation stages in CVID and IgAD.

Material and methods

Peripheral blood was obtained from 80 patients with IgAD (27 males and 53 females; aged 18-70, median 33 years), 48 patients with CVID (19 males and 29 females; aged 19-75, median 45 years), and 80 healthy controls (27 males and 53 females; aged 18-74, median 32 years).

The control group was mainly represented by relatives of hospital employees and students of medicine. The patients fulfilled the European Society for Immunodeficiencies (ESID) and the Pan-American Group for Immunodeficiency's (PAGID) diagnostic criteria for CVID and IgAD [23].

More detailed characteristics of these groups were described previously [10].

T-cell analysis

Five millilitres of peripheral blood taken in ethylenediaminetetraacetic acid (EDTA) was stained with monoclonal antibodies and analysed using flow cytometry methods. Forty-five microlitres of the blood was treated with 5–10 µl of the appropriate antibodies for 30 minutes at laboratory temperature in the dark. The blood was then processed for automated lysis, stabilisation, and fixation of the sample by a TQ-PREP workstation (Beckman Coulter, Miami, USA).

Flow cytometry analysis

Immunophenotyping was performed by flow cytometric analysis (five-colour flow cytometer FC-500; Beckman Coulter with CXP software).

For the staining of T-lymphocyte differentiation stages the following antibodies were used: R-Phycoerythrin-Cyanine 7 (PC7)-conjugated anti-CD4, anti-CD8, Phycoerythrin-Texas red X (ECD)-conjugated anti-CD8, anti-CD45RO; PC5-conjugated anti-CD27, and anti-CD25 (all from Beckman Coulter). For the staining of T_{regs} we used anti-CD4 PC7, anti-CD127 PE, and anti-CD25 PC5 (Beckman Coulter).

T lymphocytes, both CD4 and CD8, were divided into naïve CD45RO⁻CD27⁺, early differentiated (ED) CD45RO⁺CD27⁺, late differentiated (LD) CD45RO⁺CD27⁻, and fully differentiated effector (FD) CD45RO⁺CD27⁻ memory T cells. In CD8⁺ cells a unique CD45RO⁻CD27^{dim} intermediate population was determined as well [22] (Fig. 1). T_{reg} cells were defined as CD4⁺CD25^{high}CD127^{low} [24] in accordance with recent studies that demonstrated that the description of T_{regs} as a CD4⁺CD25^{high}CD127^{low} phenotype correlates well with FoxP3 expression [25, 26].

Autoantibodies

Enzyme-linked immunosorbent assay (ELISA) was used for the determination of antibodies: anti-thyroid peroxidase (AESKULISA a-TPO, Aesku.Diagnostics, Wendelsheim, Germany), anti-thyroglobulin (AESKULISA a-TG, Aesku.Diagnostics), anti-tissue transglutaminase antibodies (AESKULISA tTg-G, AESKULISA tTg-A, Aesku.Diagnostics), and the rheumatoid factor (AESKULISA RF-AGM, Aesku.Diagnostics). Anti-nuclear antibodies were tested by indirect immunofluorescence (IIF) using HEp-2 cells (HEp-2 kit, Euroimmun, Lübeck, Germany); anti-gastric parietal cells, anti-reticulin, and anti-smooth muscle antibodies were determined using the rat liver/kidney/stomach IIF kit (Autoantibodies RL/RK/RS kit, Orgentec, Mainz, Germany).

Detection of CMV-specific antibodies

IgG anti-CMV antibodies were determined by ELISA using the EIA CMV IgG kit (Test-Line, Brno, Czech Republic). The cut-off of positivity was used upon the manufacturer's instruction.

Statistical analysis

Statistical significance was determined using a non-parametric Mann-Whitney test for comparisons of two independent samples. A standard level of statistical significance $p = 0.05$ was used. The results are expressed as median (fifth to 95th percentile). All analyses were performed using Statistica 9.1 software (StatSoft Inc., Tulsa, USA).

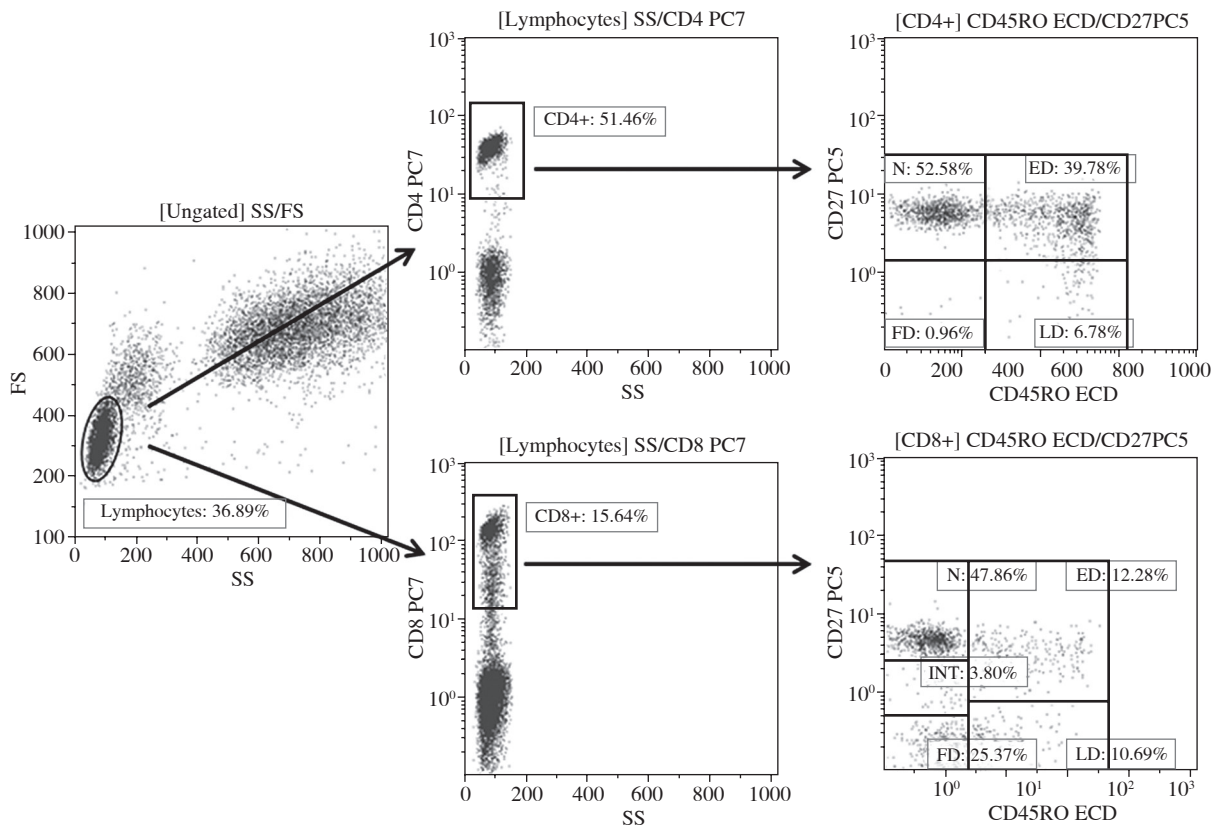


Fig. 1. Gating strategy for T-cell subsets. Gating is shown for a representative healthy donor. N naïve cells (CD45RO⁻CD27⁺), ED early differentiated (CD45RO⁺CD27⁺), LD late differentiated (CD45RO⁺CD27⁻), FD fully differentiated effector (CD45RO⁻CD27⁻), INT intermediate (CD45RO⁻CD27^{dim}) memory T cells

and GraphPad Prism 5.04 software (GraphPad software Inc., La Jolla, USA).

The study was approved by the Ethics Committee of St. Anne’s Faculty Hospital in Brno. Informed consent was obtained from all investigated individuals before the samples were drawn.

Results

Late T-lymphocyte differentiation stages are increased in IgAD and CVID patients

Comparing CVID patients to control persons, an increase of CD4⁺ and CD8⁺ late differentiated memory cells ($p < 0.001$ and $p < 0.001$, respectively), CD4⁺ early differentiated memory cells ($p < 0.001$), and a decrease of CD8⁺ intermediate memory cells ($p = 0.001$) and also of CD4⁺ and CD8⁺ naïve cells ($p < 0.001$ and $p < 0.001$, respectively) was found. In patients with IgAD a significant increase of both CD4⁺ and CD8⁺ late differentiated memory cells ($p = 0.006$ and $p < 0.001$, respectively) and a decrease in

CD4⁺ naïve cells ($p = 0.026$) were documented as compared to healthy control persons (Fig. 2).

Frequency of CMV-specific antibodies is increased in IgAD patients

Since previous studies showed increased numbers of terminally differentiated memory T cells in patients with CMV infection [27, 28], we determined CMV IgG antibodies in the sera of control persons and patients with IgAD. Using IgG as a marker of CMV positivity, 40 IgAD patients (aged 18-70, median 37.5 years) and 27 control persons (aged 19-74, median 39 years) were seropositive while 38 IgAD patients (aged 18-54, median 26.5 years) and 52 controls (aged 18-71, median 28 years) were seronegative, the difference between IgAD patients and controls being significant ($p = 0.030$, chi-square test).

We observed an increase in late differentiated CD8⁺ cells in seropositive IgAD patients compared to seropositive control persons ($p = 0.005$), while there were no significant differences when comparing other subsets in seropositive IgAD patients to seropositive controls. When

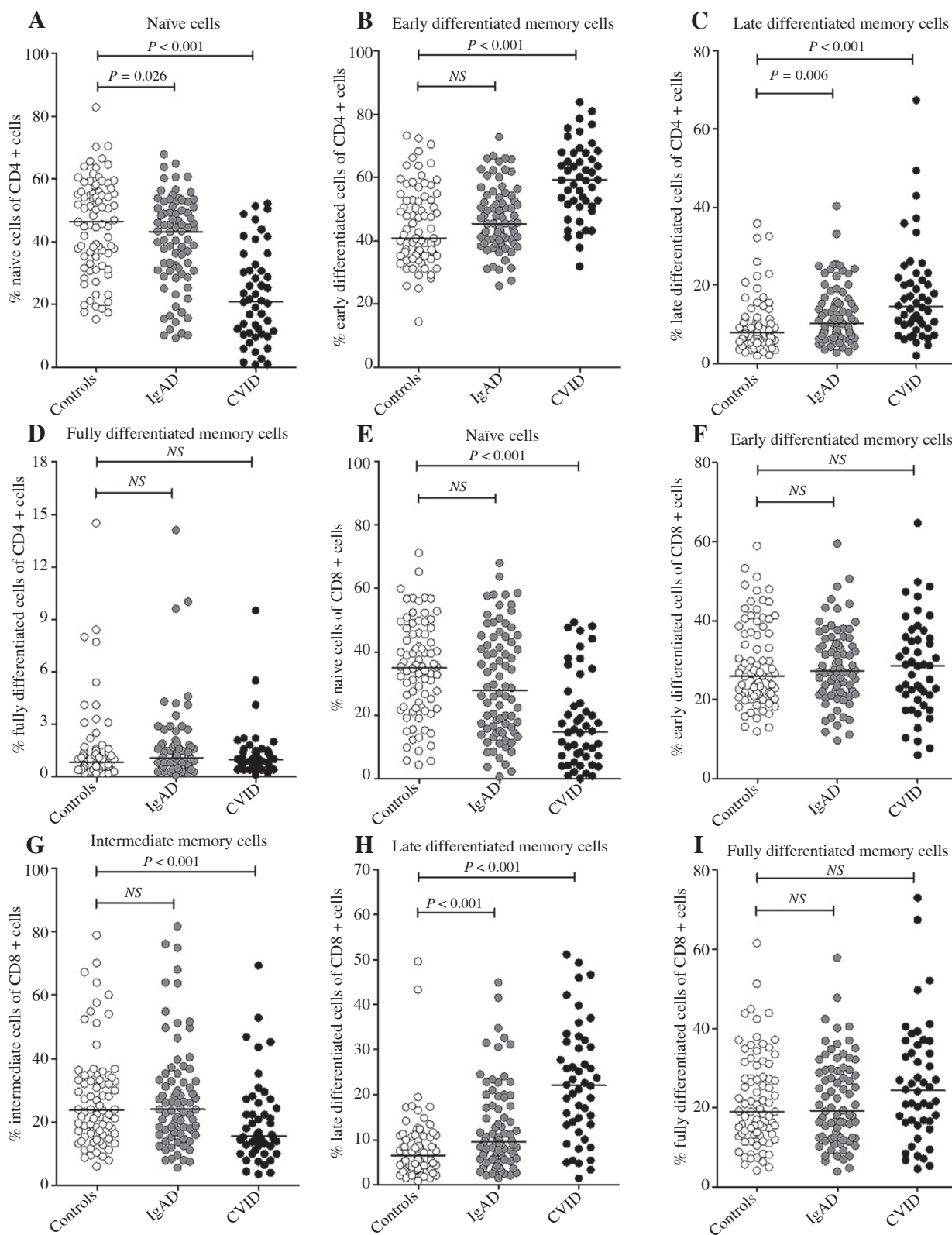


Fig. 2. Distribution of T-lymphocyte differentiation subsets in control persons and patients with IgAD and CVID. Naïve (CD45RO⁺CD27⁺) CD4⁺ and CD8⁺ cells (A, E), early differentiated memory (CD45RO⁺CD27⁺) CD4⁺ and CD8⁺ cells (B, F), intermediate memory (CD45RO⁺CD27^{dim}) CD8⁺ cells (G), late differentiated memory (CD45RO⁺CD27⁻) CD4⁺ and CD8⁺ cells (C, H), fully differentiated memory (CD45RO⁻CD27⁻) CD4⁺ and CD8⁺ cells (D, I). Horizontal lines indicate group medians; intergroup differences were analysed using Mann-Whitney test

we compared all CD4⁺ and CD8⁺ subsets in seronegative IgAD patients to the seronegative control group, we found only a very slight decrease of CD8⁺ fully differentiated memory cells ($p = 0.042$) in IgAD patients; no significant changes in other subsets were shown. When we compared CMV-seropositive to CMV-seronegative IgAD patients, we found a decrease of both CD4⁺ and CD8⁺ naïve cells (in both cases $p < 0.001$), a decrease in CD8⁺ early differentiated memory cells ($p = 0.030$), an increase in CD4⁺ and CD8⁺ late differentiated (in both cases $P < 0.001$) and fully differentiated ($p = 0.038$; $p < 0.001$, respectively) memory cells. Similar findings were obtained when comparing seropositive to seronegative controls: a decrease of both CD4⁺ and CD8⁺ naïve cells ($p = 0.041$; $p = 0.013$, respectively), and an increase in CD4⁺ and CD8⁺ late differentiated ($p < 0.001$; $p < 0.001$, respectively) and fully differentiated ($p = 0.002$; $p = 0.045$, respectively) memory cells in seropositive controls (Table 1).

Age dependence of T-cell differentiation stages

Correlating age and T-cell differentiation stages in control persons, we observed a decrease of CD4⁺ and CD8⁺ naïve cells with age ($r = -0.326$, $p = 0.003$, $r = -0.445$, $p < 0.001$, respectively), while CD4⁺ early differentiated ($r = 0.271$, $p = 0.015$) and CD8⁺ late differentiated ($r =$

0.335 , $p = 0.002$) memory cells increased. These changes were more pronounced in IgAD patients with a decrease of both CD4⁺ and CD8⁺ naïve ($r = -0.598$, $p < 0.001$; $r = -0.632$, $p < 0.001$, respectively) and an increase of late differentiated memory cells ($r = 0.4653$, $p < 0.001$; $r = 0.405$, $p < 0.001$, respectively) CD4⁺ and CD8⁺ cells, CD4⁺ early differentiated ($r = 0.537$, $p < 0.001$) and CD8⁺ fully differentiated ($r = 0.472$, $p < 0.001$) memory cells. In CVID patients a significant age-dependent correlation was observed in CD4⁺ naïve and late differentiated cells ($r = -0.468$, $p < 0.001$; $r = 0.401$, $p = 0.005$, respectively) and CD8⁺ naïve cells ($r = -0.461$, $p = 0.001$).

T_{reg} cells are decreased in CVID but not IgAD patients

The frequency of T_{reg} cells is shown in Fig. 3. We documented a decrease of T_{reg} cells in patients with CVID compared to controls ($p < 0.001$). Interestingly, we observed an increase of T_{reg} cells in IgAD patients compared to control persons ($p = 0.039$). We did not observe any significant changes in the frequency of T_{reg} cells when we compared 28 IgAD patients with the presence of autoantibodies to 52 patients without the presence of autoantibodies ($p = 0.912$) or to 31 healthy donors with autoantibodies

Table 1. Frequency of T-cell subsets in CMV⁺ and CMV⁻ IgAD patients and healthy donors

	Controls CMV+ age (median) = 39 n = 27	Controls CMV- age (median) = 28 n = 52	IgAD CMV+ age (median) = 37.5 n = 40	IgAD CMV- age (median) = 26.5 n = 38	CMV+ IgAD vs. C p	CMV- IgAD vs. C p	IgAD CMV+ vs. CMV- p	C CMV+ vs. CMV- p
CD4+CD45RO-CD27+ (naïve)	38.5 (17.4-61.6)	51.2 (21.1-66.4)	32.7 (10.2-53.5)	48.5 (15.7-63.7)	0.086	0.590	< 0.001	0.041
CD4+CD45RO+CD27+ (ED)	45.4 (31.1-60.5)	40.3 (29.1-68.2)	47.4 (31.0-65.8)	42.8 (31.0-66.0)	0.133	0.452	0.132	0.967
CD4+CD45RO+CD27- (LD)	11.0 (4.8-32.5)	7.0 (2.8-14.0)	15.3 (7.1-29.2)	7.0 (3.0-18.2)	0.143	0.407	< 0.001	< 0.001
CD4+CD45RO-CD27- (FD)	1.2 (0.4-8.0)	0.7 (0.2-4.1)	1.3 (0.4-9.8)	1.0 (0.2-2.7)	0.740	0.155	0.038	0.002
CD8+CD45RO-CD27+ (naïve)	26.5 (5.6-50.8)	37.1 (15.0-57.0)	17.4 (3.1-47.7)	40.1 (8.5-63.6)	0.065	0.551	< 0.001	0.013
CD8+CD45RO- CD27dim (INT)	6.8 (2.3-13.5)	5.6 (2.4-16.0)	5.2 (2.0-15.3)	6.6 (2.1-16.0)	0.292	0.354	0.163	0.563
CD8+CD45RO+CD27+ (ED)	25.5 (13.1-47.6)	26.8 (15.7-51.0)	26.7 (12.3-43.1)	32.4 (18.8-50.5)	0.919	0.325	0.030	0.469
CD8+CD45RO+CD27- (LD)	10.7 (4.8-43.3)	5.2 (1.5-11.6)	17.2 (6.9-38.2)	6.8 (2.0-14.9)	0.005	0.156	< 0.001	< 0.001
CD8+CD45RO-CD27- (FD)	26.5 (7.3-43.7)	17.0 (6.2-43.8)	27.1 (10.9-45.0)	12.8 (4.7-32.6)	0.650	0.042	< 0.001	0.045

CMV-seropositive (CMV⁺), CMV-seronegative (CMV⁻). N naïve cells (CD45RO⁻CD27⁺), ED early differentiated (CD45RO⁺CD27⁺), LD late differentiated (CD45RO⁺CD27⁻), FD fully differentiated effector (CD45RO⁺CD27⁻), INT intermediate (CD45RO⁺CD27^{dim}) memory T cells. Healthy donors (C). The results are expressed as median (5th to 95th percentiles). The p value was obtained by the Mann-Whitney U test

($p = 0.509$) or the whole control group (with and without presence of autoantibodies) ($p = 0.152$).

Discussion

Due to the previously described activation of the T-lymphocytes immune system in patients with CVID we determined the degree of T-cell activation by the presence of differentiation stages of T-lymphocyte memory cells. The same T-cell differentiation stages were also determined in IgAD patients.

In IgAD patients we found a higher frequency of late differentiated memory cells, in both CD4⁺ and CD8⁺ cells. In patients with CVID the changes were more extensively expressed. Comparing CVID patients to controls, besides the increase of both CD4⁺ and CD8⁺ late differentiated cells, an increase of early differentiated CD4⁺ cells and a decrease of both CD4⁺ and CD8⁺ naïve cells were observed. Loss of both CD4⁺ and CD8⁺ naïve cells (defined as CD45RA⁺CCR7⁺ or CD45RA⁺CD62L⁺) had also been described by Bateman *et al.* [29] and Giovannetti *et al.* [30], who proposed that the level of CD4⁺ naïve T cells might be considered as a parameter essential for classifying CVID due to its strong correlation with the severity of the disease and association with the “Freiburg” classification [31] based on B-cell differentiation markers.

This study showed in both IgAD and CVID patients an increase of terminally differentiated CD8⁺ cells, which are also supposed as a marker of virus infection-associated activation and memory maturation [22, 32, 33]. The link between CD8⁺ memory differentiation and immune activation was previously described [34]. This points to similarities between CVID and IgAD as observed in B-cell subpopulations [10]. This result is in contrast to the study by Bateman *et al.* [29], who did not find significant differences in any differentiated subset of T cells in the selective IgA-deficient group.

Several studies published an increase of terminally differentiated T cells in patients with HIV, leukaemia, or CVID, who suffered from CMV infection [22, 28, 33]. As our study showed an increase in the late differentiated memory cells in IgAD patients, we aimed to answer the question of whether the presence of CMV infection in IgAD patients was responsible for the above-mentioned observation. Therefore, we compared both CMV-seronegative and CMV-seropositive IgAD patients to CMV-seronegative and CMV-seropositive control persons, and found only an increase of CD8⁺ late differentiated memory cells in CMV-seropositive IgAD patients. With the exception of a slight decrease of fully differentiated memory cells in IgAD patients, all other comparisons of the differentiated subsets between seropositive or seronegative groups were not significant. We suggest that the differences between the IgAD and the control groups can be, at least partially, associated with the increased CMV seropositivity in IgAD patients.

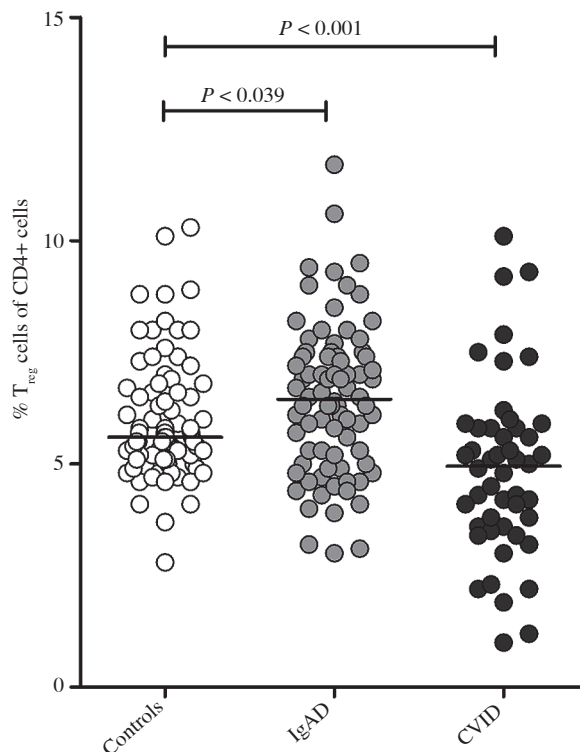


Fig. 3. Frequency of Treg cells in control persons and patients with IgAD and CVID. Horizontal lines indicate group medians; intergroup differences were analysed using Mann-Whitney test

The observation of the increase of CMV positivity in our IgAD patients was unexpected and, to our knowledge, no similar study was published. Although the groups of IgAD patients and control persons examined in our study were very similar as regards their age and sex, we cannot exclude a bias due to different socioeconomic status of the patients and control persons [35, 36] because our healthy donors were obtained mainly from the hospital staff, students of medicine, and relatives of the staff of our department, while the IgAD patients can be considered as a general, highly unselected, population. This finding deserves further confirmation.

T_{reg} cells play a critical role in suppressing the response of the immune system to self-antigens [37]. Previous studies showed decreased numbers of T_{regs} in CVID [14, 16], mainly in patients with autoimmune diseases [38]. We confirmed the decrease of T_{reg} cells in patients with CVID described previously. In contrast to CVID, surprisingly, there were increased numbers of T_{regs} in IgAD patients compared to control persons. Our observation is in agreement with the similar tendency recently published for IgAD patients by Bateman *et al.* [29] but is contrary to Soheili *et al.* [39], who described a decrease of T_{reg} cells in 26 paediatric pa-

tients of Iranian origin. Based on the previously observed expansion of T_{reg} cells in patients with chronic infections [40, 41], we suggest that the increase of T_{regs} may be associated with chronic immune activation in IgAD patients. You *et al.* in a previous study [42] described a decrease in the frequency of T_{reg} cells in CVID patients with autoimmunity compared both to patients without autoimmunity and healthy donors. Using this approach, we did not observe any similar findings in IgAD patients.

Our observation shows that terminally differentiated memory T cells are increased not only in CVID but also in IgAD patients. The increase in IgAD patients may be related to a different frequency of CMV infection in these persons. The observation of a higher frequency of CMV infection in IgAD patients deserves further study.

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