doi: 10.1111/j.1365-3083.2010.02397.x

Flow Cytometry Study of Blood Cell Subtypes Reflects Autoimmune and Inflammatory Processes in Autoimmune Polyendocrine Syndrome Type I

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Received 7 January 2010; Accepted in revised form 27 February 2010

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Abstract

Autoimmune polyendocrine syndrome type I (APS I) is a recessive disorder caused by mutations in the autoimmune regulator (*AIRE*) gene. AIRE is expressed in medullary epithelial cells where it activates transcription of organ-specific proteins in thymus, thereby regulating autoimmunity. Patients with APS I have, in addition to autoimmune manifestations in endocrine organs, also often ectodermal dystrophies and chronic mucocutaneous candidiasis. The aim of this study was to characterize immune cell subpopulations in patients with APS I and their close relatives. Extensive blood mononuclear cell immunophenotyping was carried out on 19 patients with APS I, 18 first grade relatives and corresponding sex- and age-matched healthy controls using flow cytometry. We found a significant relative reduction in T helper cells coexpressing CCR6 and CXCR3 in patients with APS I compared to controls (mean = 4.10% versus 5.94% respectively, $P = 0.035$). The pools of $CD16^+$ monocytes and regulatory T cells (Tregs) were also lower in patients compared with healthy individuals (mean = 15.75% versus 26.78%, $P = 0.028$ and mean = 4.12% versus 6.73%, $P = 0.029$, respectively). This is the first report describing reduced numbers of $CCR6^+CXCR3^+$ T helper cells and $CD16^+$ monocytes in patients with APS I We further confirm previous findings of reduced numbers of Tregs in these patients.

Introduction

Autoimmune polyendocrine syndrome type I (APS I) (OMIM 240300) is a rare autosomal recessive disorder characterized by gradual development of autoimmune disease of different endocrine and ectodermal organs and, in addition, chronic mucocutaneous candidiasis (CMC). The most common endocrine manifestations are hypoparathyroidism and autoimmune Addison's disease. The disease is characterized by autoantibodies against several defined antigens, most often tissue-specific enzymes with important functions in the affected tissues. However, tissue destruction is thought to be mediated primarily by autoreactive T cells. The aetiology of the persistent *Candida* infections has been an unsolved puzzle. However, the recently described autoantibodies against IL-17 and IL-22 may provide a new and provocative explanation for CMC; it is caused by autoimmunity, not by an immune defect *per se* [1, 2].

APS I is caused by mutations in the gene autoimmune regulator (*AIRE*), which is involved in promoting expression of tissue-specific proteins in the thymus [3–5]. This expression seems to be important to delete autoreactive T cell clones. In patients with APS I, elevated levels of autoreactive clones are thought to be released into the periphery with the potential to cause organ-specific autoimmunity. Moreover, the lack of AIRE disturbs thymic microarchitecture [6] and the local homoeostasis that can lead to impaired thymic development of cells with immunoregulatory functions like regulatory T cells (Tregs) and natural killer T (NKT) cells. Finally, Anderson *et al.* have reported on extrathymic Aire-expressing cells in mice which are capable of expressing self-antigens and can delete autoreactive T cells [7]. Similar cells have now also been found in human lymph nodes [8]. The role of AIRE in peripheral tolerance remains to be defined.

Only few and conflicting studies of immune cell subsets have been performed in relatively small cohorts of

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patients with APS I and *AIRE* mutation carriers. Reduced number of invariant NKT (iNKT) cells, but normal natural killer (NK) cell counts, were recently reported in patients with APS I [9]. An early study on three patients with APS I reported on a range of immunological abnormalities in both patients and their close relatives, including increase in serum IgM, IgG and IgE and lack of IgA in some individuals. Abnormal suppressor T cell function (as tested by lymphocyte response to phytohemagglutinin after exposure to concanavalin A) and an elevated B-cell level (tested by a technique involving polyvalent antihuman Ig serum) were also seen [10]. In agreement, Sediva *et al.* reported on marked elevation of IgM in a study comprising four patients with APS I [11]. The ratio CD4:CD8 has been found both elevated and decreased in different studies [12–17] and various results have been reported for the level of $CD19^+$ B cells in patients [15, 17, 18]. Additionally, increased monocyte numbers have been reported in the blood of APS I patients and in non-APS I patients with persistent *Candida* infections [19, 20].

Reduced levels or deficiency in the function of cells with immunoregulatory or suppressive nature may contribute to autoimmune pathology. Perniola *et al.* [16] performed immunophenotyping of 11 patients with APS I and found a significantly increased level of $CD8^+CD11b^+$ cells, which is thought to be a suppressor cell subset. Tregs, characterized by expression of the forkhead box P3 (FoxP3) protein and CD25, but lacking the IL-7 receptor CD127, have suppressive functions which enable them to control inflammatory and autoimmune responses (for review see [21]). In a cohort of 26 Finnish patients with APS I, normal numbers of $CD4^+$CD25high cells were found, but less FOXP3 mRNA was expressed, both in the CD25high subset and in the total T-cell population. These alterations were accompanied by lower suppressive function towards effector cell proliferation than in healthy controls [22]. However, the frequency of $CD4^+CD25^+$ cells, which also contain activated cells, was much higher in patients with APS I than in controls [16].

The reported frequency of circulating immune cell subpopulations varies in different studies, and commonly only a limited number of patients with APS I has been studied. We here aimed to study a wide range of immune cell subsets relevant for characterizing thymic output of cells with regulatory functions as well as peripheral dysregulation of effector/memory cell subsets in a relatively large number of patients with APS I and their close relatives.

Methods

Patients and control subjects. Nineteen Norwegian patients with APS (10 men, 9 women; mean age 34.1 years; range 18–58) and appropriate age- and sex-matched healthy controls (Ctrl 1; mean age 36.8 years, range 18–61) were included for immunophenotyping. We also included 18 close relatives (8 men, 10 women; mean age 47.2 years, range 18–70) and age- and sex-matched controls (Ctrl 2; mean age 43.2 years, range 18–61). Two of the included relatives had self-reported autoimmune diseases, namely Sjøgren's syndrome and coeliac disease, respectively. Not all subjects were examined for all immune cell subsets. Serum samples for autoantibody analyses were available from 37 Norwegian patients with APS I and 35 close relatives (parents, siblings or other close family members). All patients had mutations and/or deletions in both *AIRE* alleles [23, 24] and most of the patients are reported on earlier [24]. All included patients signed a written consent form and were recruited via the Norwegian Registry for organ-specific autoimmune diseases (ROAS). Family members of patients with APS I were recruited via the patients. Healthy controls were recruited from the blood bank at Haukeland University Hospital. Demographics of the patients and relatives and their *AIRE* mutations are summarized in Table S1. The study was approved by the local ethics committee.

Flow cytometry. EDTA-Blood was collected, and peripheral blood mononuclear cells (PBMC) were isolated using Lymphoprep (Axis-Shield PoC AS, Oslo, Norway). We incubated 2×10^5 PBMC in 100 μl of PBS with labelled monoclonal antibodies [mAbs; Beckton Dickinson (BD) Biosciences] to human cell markers: CD3 (PE-Cy7; SK7 and PerCP; SK7), CD4 (PerCp; clone SK3 and PE-Cy7; clone SK3 and FITC; RPA-T4), CD5 (PE; UCHT2), CD8 [FITC; RPA-T8 and PE-Cy7; RPA-T8 and PE, RPA-T8 and PE (SK1)], CD11b/Mac-1 (PE; ICRF44), CD11c (APC; B-ly6), CD14 (PE-Cy7; M5E2 and PerCp; MφP9), CD16 (FITC; 3G8), CD19 (FITC, HIB19), CD25 (APC; 2A3 and PE; 2A3), CD28 (APC; CD28.2), CD45RA (FITC; HI100), CD45RO (APC; UCHL1), CD56 (APC; B159), CD62L (PE, Dreg 56), CD127 (biotin; HIL-7R-M21), CD161 (FITC; clone DX12), invariant NK T (PE, 6B11), FoxP3 (Alexa Fluor 647; 259D/C7), CCR6/CD196 (PE; clone 11A9), CXCR3/CD183 (APC; clone 1C6/CXR3) and CCR4 (biotin-clone 1G1) followed by anti-biotin (APC) from Miltenyi Biotech (Bergisch Gladbach, Germany). The following antibodies were purchased from Miltenyi Biotech: TCR Vbeta 11 (FITC), TCR Valpha24 (PE) and anti-biotin (APC; Bio3-18E7). Fcγ-blocking antibodies (Miltenyi Biotech) were added to the assays where B cells and monocytes were examined. After washing, cells were fixed in 1% paraformaldehyde, and four markers were analysed simultaneously using FACS Calibur (BD BioSciences, San Jose, CA, USA) and FlowJo version 7.2.5 (Tree Star, Ashland, OR, USA).

Whole blood enumeration of type-1 myeloid dendritic cells (MDC1), type-2 myeloid dendritic cells (MDC2) and plasmacytoid dendritic cells (PDC) was carried out using the human blood dendritic cell enumeration kit from Miltenyi Biotech, following the instructions from the manufacturer. CD303 (also called CLEC4C or

BDCA-2) identified PDC, CD1c (BDCA-1) detected MDC1 whereas MDC2 cells was counted based on their CD141 (BDCA-3/thrombomodulin) expression. For DC subtyping, at least 300,000 cells were analysed.

Assay of autoantibodies in patients with APS I and their relatives. Autoantibodies against organ-specific autoantigens [21-hydroxylase (21OH), side-chain cleavage enzyme (SCC), glutamic acid decarboxylase-65 (GAD-65), NACHT leucine-rich-repeat protein 5 (NALP5), aromatic L-amino acid decarboxylase (AADC) and type I interferons (IFN-ω)] were assayed using radioimmunoassay based on the proteins expressed by *in vitro* transcription and translation (Promega, Madison, WI, USA) as described earlier [25].

Statistics. The differences between patients and age/-sex-matched controls (Ctrl I) and between relatives and age/sex-matched controls (Ctrl 2) were calculated using the Mann–Whitney test in SPSS v.15 (SPSS Norway AS, Oslo, Norway) and/or GRAPHPAD v.5 (GraphPad Software Inc., La Jolla, CA, USA). *P*-values below 0.05 were considered statistically significant.

Results

Patients with APS I exhibit a reduced frequency of Tregs, but not NKT cells

Results of the immunophenotypical analysis of peripheral blood cell subpopulations, as proportions in the lymphocyte or Th-cell compartments, are summarized in Table S2. We first sought to confirm published dysregulations in the cell populations with immune regulatory function. Their deficiency could contribute to the autoimmune features of patients with APS I and reflect the thymic dearrangements in producing these cells. Indeed, patients displayed significantly lower proportions of Tregs, as identified by analysis of $CD4^+CD25^+FoxP3^+$ and $CD3^+CD4^+CD25^+CD127^-$ cells in the Th compartment in comparison with control individuals ($P = 0.029$ and $P = 0.028$ respectively) (Fig. 1). When calculating the number of $CD4^+CD25^+FoxP3^+$ relative to lymphocyte count, the frequencies in patients with APS I and controls were the same. However, the $CD127^-$ Treg compartment, which also represents Tregs, remained significantly reduced compared to controls relative to lymphocytes ($P = 0.011$) (Table S2). APS I relatives also had lower number of these cells, although only borderline significant ($P = 0.050$). Invariant CD1d-restricted NKT-cells (iNKT), which are supposed to play an inhibitory role in autoimmune diseases, were identified with the help of several characteristic surface markers as Vα24, Vβ11, CD161 and the Invariant NKT-molecule (Table S2). In contrast to Tregs, we did not observe any alterations in iNKT cells in our patients with APS I. Contrary to a previous report [16], the suppressor subset characterized by the markers $CD8^+CD11b^+CD28^+$ revealed no significant differences between our studied groups.

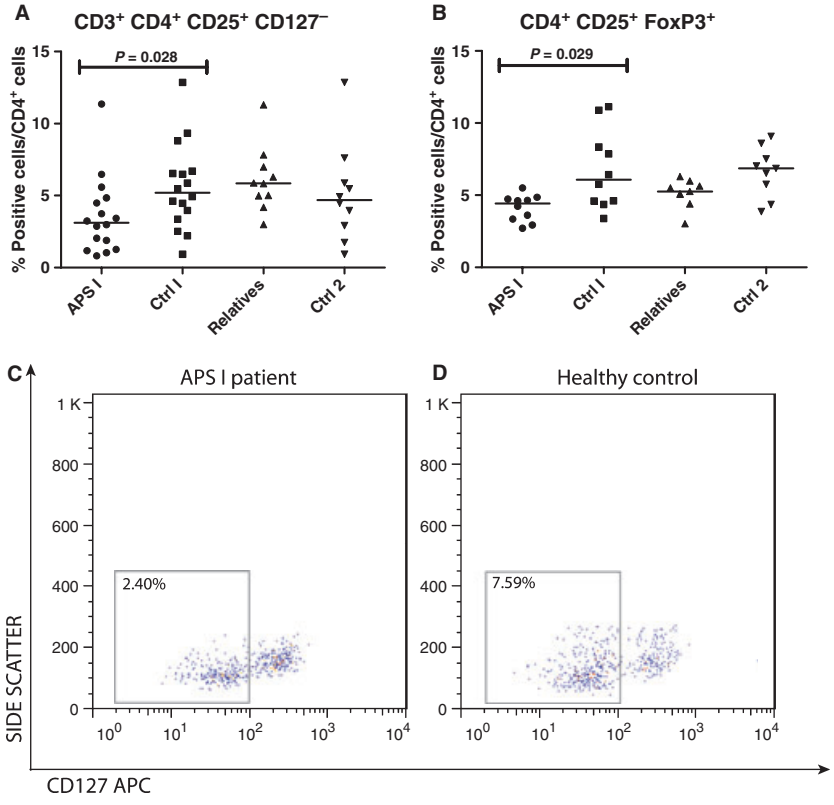

Figure 1 Distribution of regulatory T cells (Tregs) in patients with autoimmune polyendocrine syndrome type I (APS I), their relatives and healthy controls. (A) Percentage of $CD3^+CD4^+CD25^+CD127^-$ cells with respect to $CD4^+$ lymphocytes, (B) Percentage of $CD4^+CD25^+FoxP3^+$ cells with respect to $CD4^+$ lymphocytes, (Median values are shown as a line in the graphs. Only statistical significant *P*-values are given in the plots.) (C and D) FLOWJO graphs representing Tregs ($CD3^+CD4^+CD25^+CD127^-$ cells) from an patient with APS I (C) and a healthy control (D). The diagrams show cells first gated on lymphocytes, then on $CD3^+CD4^+$ cells, then on $CD25^+$ cells. The *x*-axis represents CD127 APC-fluorescent cells whereas the *y*-axis represents side-scatter. The percentage of CD127 low cells in the $CD3^+CD4^+$ cell cohort is given in the respective gates.

Patients with APS I have reduced proportion of CCR6 and CXCR3 coexpressing Th-subset

Further, we analysed several effector/memory T cell subtypes in patients with APS I and their relatives in comparison with control individuals. We first confirmed that the percentages of T cells, T helper cells ($CD4^+$) and cytotoxic T cells ($CD8^+$) were similar in patients and controls (Table S2). Unexpectedly, we observed that APS I family members had significantly decreased frequency of memory Th cells ($CD4^+CD45RA^-CD45RO^+$) compared to healthy controls ($P = 0.023$) (Table S2, Fig. 2). Next, we sought to compare the frequency of Th cell subsets with different homing properties according to differentially expressed chemokine receptors. CCR6 and CXCR3 were of particular interest as $CCR6^+$ cells are attracted to epithelial surfaces by CCL20 and can be involved in protection against CMC; CXCR3-expressing cells are attracted to inflammatory tissues by binding to interferon-induced chemokines CXCL9-11 [26, 27]. We did not find alterations in the proportions of $CD4^+CD45RA^-CCR4^+CCR6^+$ lymphocytes that have been reported to contain IL-17A-secreting Th17 cells (Table S2). In contrast, the percentage of CCR6 and CXCR3 coexpressing Th subpopulation, which includes among others IFNγ and IL-17A coproducing cells, was significantly decreased in patients with APS I ($P = 0.035$) (Fig. 3) [26].

Patients with APS I have reduced frequency of the Fc-receptor CD16 in their monocyte compartment

Next, we examined the abundance of myeloid cell subsets in patients with APS I. DC can be subdivided into several undergroups, here separated into MDC1, MDC2 and

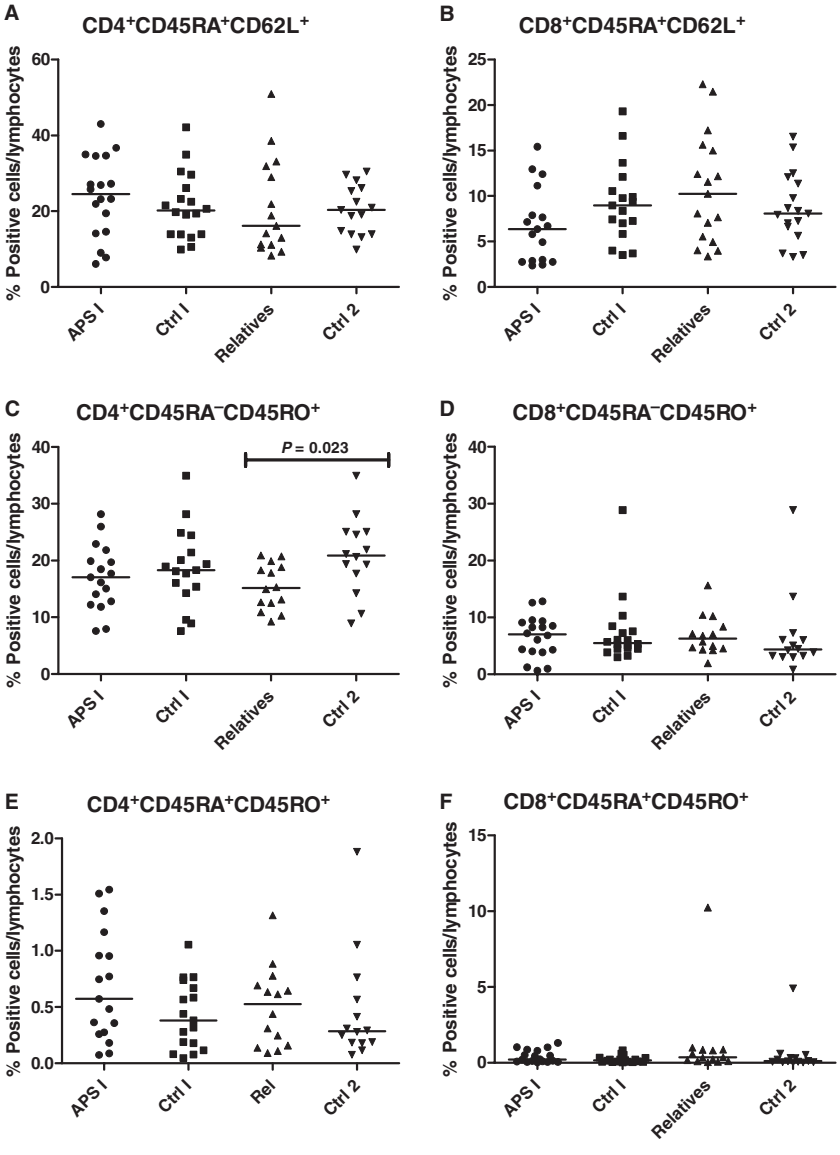

Figure 2 Distribution of peripheral naive and memory T-cell subpopulations in patients with autoimmune polyendocrine syndrome type I, relatives and controls. (A) The percentage of Th ($CD4^+$) naive cells with respect to lymphocytes ($CD4^+CD45RA^+CD62L^+$/lymphocytes). (B) The percentage of Tc ($CD8^+$) naive cells with respect to lymphocytes ($CD8^+CD45RA^+CD62L^+$/lymphocytes). (C) The percentage of Th memory cells with respect to lymphocytes ($CD4^+CD45RA^-CD45RO^+$/lymphocytes). (D) The percentage of Tc memory cells with respect to lymphocytes ($CD8^+CD45RA^-CD45RO^+$/lymphocytes). (E) The $CD45RA^+CD45RO^+$ double producing cell compartment of Th ($CD4^+$) cells relative to lymphocytes. (F) The $CD45RA^+CD45RO^+$ double producing cell compartment of Tc cells ($CD8^+$) relative to lymphocytes. (A–F) Lymphocyte count refers to cells corresponding to the lymphocyte population in the forward and side-scatter plot. (Median values are shown as a line in the graphs. Only statistical significant *P*-values are given in the plots.)

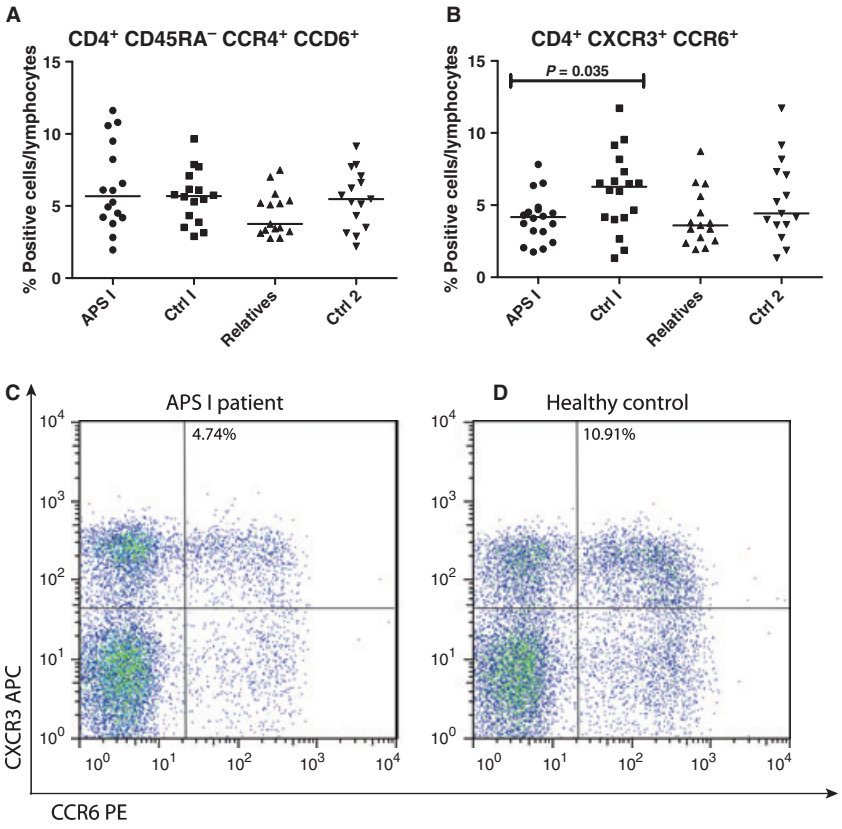

Figure 3 Distribution of blood Th17 cells in patients with autoimmune polyendocrine syndrome type I (APS I), relatives and healthy controls. (A) The percentage of $CD4^+$ $CD45RA^-CCR4^+CCR6^+$ cells relative to lymphocyte number. (B) The percentage of $CD4^+CCR6^+CXCR3^+$ cells relative to lymphocyte number, (Median values are shown as a line in the graphs. Only statistical significant *P*-values are given in the plots.) (C and D) Representative presentations of graphs showing levels of $CD4^+$ T cells coexpressing CXCR3 and CCR6 for an patient with APS I (C) and a healthy blood donor (D). The diagrams show cells first gated on lymphocytes, then on $CD4^+$ cells. The *x*-axis represents $CCR6^+$ PE-fluorescent cells whereas the *y*-axis represents $CXCR3^+$ APC coloured cells. The percentage of $CCR6^+CXCR3^+$ cells in the $CD4^+$ cell cohort is given in the respective gates.

PDC. PDC differ from MDC in both the expression of pattern recognition receptors, cytokine receptors, cytokines and migration capability [28]. MDC1 are capable of differentiating to Langerhans cells whereas MDC2 cells are not [29]. No differences in the frequencies of dendritic cells were seen in our study (Table S2). Contrary to previous reports, the proportion of monocytes, as determined by CD14 expression in the compartment of live cells purified by Lymphoprep, showed no deviations between patients, controls and relatives. However, large individual variations were seen. When characterizing the monocyte subpopulations, we found that relatives had trends towards less $CD14^+CD11b^+$ than their control group ($P = 0.053$) (Table S2). The integrin CD11b is known to be involved in cell adhesion and movement to inflammatory sites [30]. Yet another monocyte subpopulation of interest is the $CD14^+CD16^+$ circulating pool of cells which is associated with acute or chronic inflammation [31, 32]. In our cohort, we found that patients with APS I had significantly less $CD14^+CD16^+$ cells than healthy blood donors ($P = 0.028$) (Table S2, Fig. 4).

The frequencies of B cells and NK cells are unchanged in patients with APS I

APS I is characterized by high titres of a broad spectrum of autoantibodies and increased immunoglobin levels. However, the frequencies of regular B cells and $CD5^+$ B cells were unchanged in patients with APS I in comparison with healthy individuals (Table S2).

The frequency of NK cells ($CD3^-CD56^+$) was not significantly different between patients with APS I, relatives and controls. We further calculated the relative amount of subgroups of these cells. We first looked at NK cells expressing CD62L. This molecule mediates lymphocyte homing to high endothelial venules of peripheral lymphoid tissue and leucocyte rolling on activated endothelium at inflammatory sites [33]. Hence, obtaining information on the expression of CD62L on patient NK cells can indicate whether the migration of these cells is normal. However, no differences in $CD62L^+$ NK cells were found between the groups. $CD16^+$ and $CD16^-$ NK cell subsets differ in their cytokine production capacity and so also in their role in immune regulation [34]. Patients with APS I expressed less CD16 in our study, although the results did not reach statistical significance (Table S2).

The presence of IFNω autoantibodies and autoantibodies against organ-specific antigens in patients with APS I and close relatives

Thirty-seven patients with APS I and 35 close relatives (the mutational status of *AIRE* was not known for all relatives) were analysed for serum autoantibodies against several proteins known to be targeted in patients with

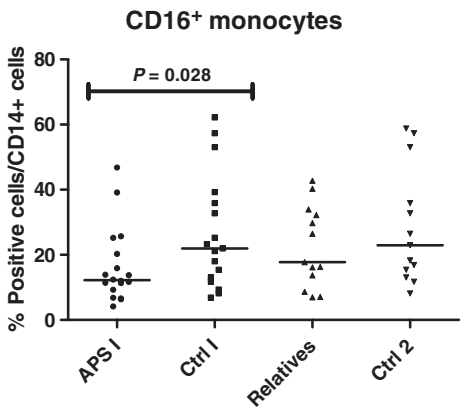

Figure 4 Distribution of $CD16^+$ monocytes in patients with autoimmune polyendocrine syndrome type I, relatives and healthy controls. Percentage of $CD14^+CD16^+$cells with respect to monocytes ($CD14^+$ cells). The cells were first gated on forward and side scatter to eliminate dead cells. Then, the cells were gated on $CD14^+$ live cells (PerCp) followed by $CD16^+$ cells (FITC). (Median values are shown as a line in the graphs. Only statistical significant *P*-values are given in the plots.)

APS I. All patients had antibodies against IFN-ω, and most of them also had antibodies against one or more of the other included antigens. No relatives were found to exhibit autoantibodies against autoantigens found in APS I (Table 1).

Discussion

We have conducted a broad immunophenotyping study of relatively large cohorts of patients with APS I and relatives. Analysis of our patients with APS I revealed a few cellular abnormalities, some of which are novel. However, the distinctive changes in blood immune cell composition in patients with APS I were not observed in their family members.

Norwegian patients with APS I exhibited reduced relative numbers of Tregs. These cells are known to be crucial for avoiding pathological autoimmunity. Mutations in FoxP3 cause the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome which is characterized by development of multiple autoimmune disorders in affected individuals. Aberrations in function of Tregs or their decreased numbers have been found in several autoimmune conditions, including early onset type 1 diabetes, APS II and in patients with the common variable immunodeficiency syndrome with autoimmunity [35–37]. The finding of lower levels of FoxP3-positive cells in the T-cell pool in patients with APS I has also been shown before [22]. We confirm and extend these previous observations using another marker for regulatory T cells, namely the $CD4^+$ cell population with low CD127 expression [38]. Kekaleinen *et al.* revealed in their study that Tregs in patients with APS I do not function properly and that they have alterations in their TCR repertoire. All these data point towards a role of Tregs in the pathogenesis of APS I. We speculate that AIRE is involved in the development of Tregs, either in the thymus or in the lymph nodes where AIRE is also expressed [7, 8]. Thymic abnormalities could potentially also interfere with the proper development of iNKT cells – another type of cells with immunoregulatory properties. However, we could not confirm the previous reported decreases in iNKT [9] in Norwegian patients with APS I.

Changes in the peripherally induced effector or memory cells could also reflect the autoimmune attack on endocrine organs. The percentage of the $CCR4^+CCR6^+$ Th-cell population which includes IL-17A producing Th17 cells was unaltered in patients with APS I in our study. This is in line with a previously published study on isolated CMC [39] and our recent report of unchanged IL-17A responses in spite of severely decreased IL-17F and IL-22 responses in APS 1 patients' PBMC [1]. IL17-producing cells have been reported to be involved in protection against *Candida albicans* (reviewed in [40]). These cells are also involved in the pathogenesis of many autoimmune diseases, including psoriasis, rheumatoid arthritis and Crohn's disease [41–43]. Hence, pathological autoimmunity can be associated with an increased

Table 1 Autoantibodies in patients with autoimmune polyendocrine syndrome type I (APS I) and relatives.

Autoantigen	Positive APS I patients (N = 37)	Positive APS I relatives (N = 35)	Associated to manifestation/ diagnosis in APS I
IFN-ω	37 (100%)	0	APS I [47]
NALP-5	12 (32%)	0	Hypoparathyroidism [48]
AADC	15 (39%)	0	Hepatitis and vitiligo [49]
SCC	13 (38%)	0	Gonadal failure [50, 51]
21OH	22 (60%)	0	Addison's disease [50, 52]
GAD-65	10 (27%)	0	Intestinal dysfunction [50]

IFN-ω, interferon omega; NALP-5, NACHT leucine-rich-repeat protein 5; 21OH, 21-hydroxylase; SCC, side-chain cleavage enzyme; GAD-65, glutamic acid decarboxylase-65; AADC, aromatic L-amino acid decarboxylase.

Th17-cell response whereas a decreased function or number of these cells is correlated to CMC. The fact that patients with APS I are both susceptible for autoimmune diseases and for CMC might complicate the cellular analysis. Interestingly, we observed a significant decrease in $CCR6^+CXCR3^+$ Th-cell proportion in patients with APS I. The mechanism underlying this phenomenon could be an increased homing of these cells to inflammatory tissues by binding to interferon-induced chemokines CXCL9 and 10; hence, these cells will be found in a decreased level in the circulation. Indeed, we have previously shown increased levels of CXCL10, a CXCR3 ligand, in APS I patient's sera that is probably secreted by endothelial cells in inflamed tissues in response to IFNγ [44].

The level of different DC subpopulations did not vary between the groups. This is in agreement of what we and others have published earlier [19, 38]. The monocyte level of patients with APS I has been shown by Hong *et al.* and Perniola *et al.* [19, 45] to be increased in patients compared to controls. The monocyte frequencies of patients varied a lot in our study, and some of the patients had indeed elevated numbers of these cells. However, when comparing the group as a unifying cohort, the results did not reach significance. To speculate, it might be possible that the elevated monocyte level in patients with APS I is linked to distinct phases in the pathogenesis of the disease, or to certain manifestations, although we were unable to see any patterns to support this. Instead, we found lower levels of $CD16^+$ cells in the pool of monocytes in our APS I cohort. CD16, also termed 'FcγRIII', is a member of the Fc-receptor family (for review, see [46]). This receptor is specific for binding small IgG complexes, which should be constantly forming in APS I as they have high titres of a plethora of autoantibodies. Crosslinking CD16 can induce production of TNFα and IL1β in monocytes. It has been reported that $CD16^+$ monocytes and $CD16^-$ monocytes have the same capability of differentiating into DC, but the expression of specific DC markers like CD86, CD11a and CD11c and their potential to secrete IL-4 and proinflammatory cytokines differ [31, 32]. The downregulation of CD16 on APS I monocytes could be a result of massive immune complex binding to the receptor followed by internalization.

Our studies showed contradictory results for many immune cell subpopulations compared with earlier reports. Several of the cellular abnormalities described here or previously are most probably not the result of thymic malfunction but the reflection of longstanding autoimmunity and inflammation caused by *C. albicans* infection. As the study groups cannot be large because of the rarity of the disease, the results of immunophenotyping may depend on the duration and activity of the disease components in studied patients.

In conclusion, we here report the most comprehensive immunophenotypic study which has been published on patients with APS I and relatives. Our data suggest that patients with APS I have disturbances in the Treg compartment, less $CCR6^+CXCR3^+$ Th cells and less $CD16^+$ monocytes, which may explain their propensity for autoimmune manifestations.

Acknowledgment

We will express our gratitude to the patients, relatives and healthy controls for donating blood samples for the study. The doctors Kristian Fougner, Jens Bollerslev, Kristian Løvås and Bjørn Nedrebø are thanked for recruiting patients to the study. We will furthermore thank Hajirah Muneer, Institute of Medicine, University of Bergen, for excellent technical skills in the handling of cell samples. The study was supported by grants from Helse Vest and the European Regional Fund and Archimedes Foundation and Estonian Science Foundation grant 8358. Anette Bøe Wolff has been a post-doctoral fellow of the The Research Council of Norway.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Demographics of APS I families included in the immunophenotypic studies.

Table S2 Immunophenotyping of APS I patients, relatives and healthy controls.

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