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RESEARCH LETTER

Systemic interferon type I and B cell responses are impaired in autoimmune polyendocrine syndrome type 1

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Autoimmune polyendocrine syndrome type I (APS-1) is caused by mutations in the autoimmune regulator (*AIRE*) gene and characterised clinically by multiple autoimmune manifestations and serologically by autoantibodies against tissue proteins and cytokines. We here hypothesised that lack of AIRE expression in thymus affects blood immune cells and performed whole-blood microarray analysis (N = 16 APS-I patients vs 16 controls), qPCR verification, and bioinformatic deconvolution of cell subsets. We identified B cell responses as being downregulated in APS-1 patients, which was confirmed by qPCR; these results call for further studies on B cells in this disorder. The type I interferon (IFN-I) pathway was also downregulated in APS-1, and the presence of IFN antibodies is the likely reason for this mild overall downregulation of the IFN-I genes in most APS-1 patients.

Keywords: APS-1; AIRE; transcriptome; blood; interferon type 1

Autoimmune polyendocrine syndrome type I (APS-1) is a rare monogenic disorder with a prevalence of 1:100 000 in most populations. It is characterised clinically by the triad of autoimmune Addison's disease, hypoparathyroidism, and chronic mucocutaneous candidiasis [1,2]. The underlying cause is specific mutations in the autoimmune regulator (*AIRE*) gene, a key regulator of thymic expression of numerous self-proteins [3–5]. Consequentially, immune tolerance of developing T cells is lost, leading to tissue damage. Highly specific autoantibodies against immunoregulatory cytokines and tissue-specific antigens are typical. Notably, neutralising autoantibodies against type I interferons (IFN-I), interleukin (IL)-17, and IL−22 are specific hallmarks of APS-1 and can provide additional diagnostic support [6–9].

The rarity of APS-1 and inaccessibility of thymic tissue have severely limited immunological studies in these patients. The devastating consequence of missing AIRE in tolerance must have implications for peripheral T cells, but also B cells, supported by findings of circulating antibodies against a range of targets [10]. Although a downregulation of regulatory T cells (Tregs) levels and/or function is consistently found [11–14], reports on frequencies of T and B cell subsets have shown conflicting results [13,15–20]. Perri and

Abbreviations
APS-1, autoimmune polyendocrine syndrome type 1; AIRE, autoimmune regulator; FDR, false discovery rate; GS, gene sets; GSEA, gene set enrichment analysis; IFN, interferon; IL, interleukin; LE, leading edge; NK cells, natural killer cells; PCA, principal component analysis; PCR, polymerase chain reaction; pDC, plasmacytoid dendritic cells; RIN, RNA integrity numbers; ROAS, Norwegian registry and biobank for organ-specific autoimmune disorders; Tregs, regulatory T cells.

colleagues recently reported that B cells from APS-1 patients display impaired ability to proliferate in response to CpG *in vitro* and that patients with long-standing disease have more disturbed B cell subset repertoires than patients with less than 15 years of active (or diagnosed) disease [19]. Sng *et al* elegantly showed that the diminished Treg pool in APS-1 patients correlated with enhanced proliferation and increased expression in B cells using a surrogate marker for autoimmune potential (HEp-2-reactive antibodies) [13]. Otherwise, only minor and not verified effects on the immune repertoire have been examined, including levels of blood components outside the adaptive immune response, e.g. monocytes, dendritic cells, and natural killer (NK) cells [14,17,18,21–23], but again no large disturbances have been consistently revealed.

We here aimed to identify transcripts and immune pathways that are up/downregulated in whole blood from APS-1 patients by a global microarray transcriptomic technique and subsequent designated real-time PCR. Our data can contribute to understanding of the deficient immune function in APS-1 patients.

Methods

Ethics approval

This project was conducted in compliance with the Declaration of Helsinki and approved by the Regional Ethical Committee of Western Norway (approval numbers 2009/2555 and 2018/1417). All patients were recruited from the Registry and biobank for organ-specific autoimmune disorders (ROAS), Haukeland University Hospital, Norway, and gave written informed consent for participation. Control samples were obtained from the Haukeland University Hospital blood bank, and these individuals were anonymised when processed and analysed.

Patients and controls

Sixteen Norwegian patients with APS-1 (8 males, 8 females, mean age 35.9 years +− 16.2) and 16 Norwegian gender and age-matched controls (±5 years) were included in the blood microarray expression study. Fourteen of the same 16 patients and 4 additional APS-1 patients were included in the real-time PCR verification experiments together with 8 additional controls for a total of 18 APS-1 patients and 24 healthy controls.

The included APS-1 patients have all been reported previously, and diagnosis was always confirmed by the clinical criteria for this syndrome, *AIRE* mutational analysis and/or autoantibody screening against IFN-ω [24]. All patients were additionally analysed for antibodies against organ-specific targets known for APS-I and most for the cytokine targets IL-22, IL-17A, IL-17F, IFN-α2, and IFN-α8. None of them were on immune suppressive treatments except for glucocorticoids to restore physiological cortisol levels for patients with autoimmune Addison's disease. Data of the patients and degree of overlap between the data sets are summarised in Table 1.

Both patients and healthy individuals gave blood for RNA when they were considered to be devoid of infections. The APS-1 patients are known to be faced with frequent episodes of *Candida albicans* infections, but historic infectious states of fungi or other infections were not available.

Consent to participate

All patients were recruited from the Registry and biobank for organ-specific autoimmune disorders (ROAS), Haukeland University Hospital, Norway, and gave written informed consent for participation.

Experimental approach microarray transcriptomics

Sampling of APS-1 patients and controls was performed in standardised manners in PAXgene blood RNA tubes (PreAnalytix, Qiagen, Hombrechtikon, Switzerland) and stored at −80 °C until use. Purification of RNA was achieved by the PAXgene blood RNA kit following the instructions from the manufacturer. Samples were quality assessed by Agilent Bioanalyzer, using the Agilent 6000 Nano kit (Agilent, Santa Clara, CA, USA), providing RNA with RNA integrity numbers (RIN) above 6.0. The samples were randomly distributed into 4 batches for RNA extraction, each with 4 patients and 4 sex- and age-matched controls, and were extracted by the same person on the same day. Following the procedures from Illumina, RNA was subsequently transformed to cRNA, and these constructs were labelled, amplified, and quality-checked again by the Agilent Bioanalyzer. The cRNAs were then hybridised to 4 Illumina HumanRef-8 BeadChip microarrays, followed by washing and scanning according to the protocol. Quality control of the arrays was done by BeadStudio.

Bioinformatic approach microarray transcriptomics

Microarray expression output was analysed using *Limma* within R [25]. A list of differentially expressed genes (GRCh38p13) between patients and controls was then provided. Genes targeted by multiple probes were collapsed, and the list comprising 11 441 features was ranked according to log2 fold change and interrogated using gene set enrichment analysis (gsea) [26]. The 26 978 gene sets (GSs) identified were compiled on 1 March 2021 using the

Table 1. Characterisation of APS-1 patients.

APS-I number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Family	1		2		3		2			1	1				2							3	
AIRE mutation	1/2	3/3	3/3	3/3	4/5	3/3	3/3	1/1	1/1	1/2	1/2	6/6	1/7	3/3	3/3	3/3	6/6	1/8	1/4	3/9	3/3	3/3	1/8
AIRE mutation group	2	3	3	3	4	3	3	1	1	2	2	5	2	3	3	3							
Included in substudy																							
Microarray blood analysis	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x							
Real time PCR verification	x	x	x	x	x	x	x	x	x			x	x	x	x	x	x	x	x	x			
Autoantibodies in serum																							
IFN-ω Binding/neutralising Abs	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/NA	1/NA	1/1	1/NA	1/NA	1/1	wk/1	1	1/1
IFN-α2 Binding/neutralising Abs	1/1	1/1	1/1	1/NA	0/0	1/1	1/1	1/1	1/1	1/1	1/1	0/wk	0/0	1/1	1/NA	1/NA	1/1	1/NA	1/NA	1/1	1/wk	1/NA	1/1
Binding IFN-α8 Abs	1	1	1	1	0	1	1	1	1	1	1	wk	wk	1	NA	1	0	1	NA	1	1	1	1
IL22 Binding/neutralising Abs	1/1	1/wk	1/1	1/0	0/0	1/1	1/0	1/1	1/1	1/1	0/1	0/0	0/0	1/1	1/1	1/1	0/0	1/NA	1/NA	1/1	1/1	1/NA	1/wk
Organ-specific Abs $N = 8$	3	4	2	1	4	3	3	1	5	4	3	4	3	2	6	4	2	1	6	3	6	4	3

AIRE mutations: 1. c.769C > T; 2. c.1242_1243insA; 3. c.967-979del13; 4. c.22C > T; 5. c.402delC; 6. c.879 + G > A; 7. c.1336 T > G; 8. c.1249dupC; 9. del e1-e8. *AIRE* mutation group microarray analysis: 1. c.769C > T homozygous: Finnish; 2. C.769C > T + other: Finnish het.; 3. c.967-979del13 homozygous: 13 bp del.; 4. missense + ins/del: point mut.; 5. c.879 + 1G > A: Splice. Used to classify mutations in the microarray analysis interpretations. Autoantibodies in serum: 1: Positive. wk: very weak positive response. NA: not analysed. 0: negative. Organ-specific autoantibodies: The number represents how many of the following autoantibodies each patient has in serum (total $N = 8$): 21-hydroxylase, 17-hydroxylase, glutamic acid decarboxylase-65, side-chain cleavage enzyme, aromatic L-amino acid decarboxylase, tryptophan hydroxylase, tyrosine hydroxylase, NACHT leucine-rich-repeat protein 5.

resources described in [27]. Permutation number was deemed adequate at 1000 iterations, 8972 GSs passed the size thresholds set at >10 and <500 member genes, and for all other parameters, default values were used.

The contribution of individual genes within each GS is subsequently addressed via leading edge (LE) analyses. They identify each GS's member genes that appear in the ranked list at or before the point at which the running sum reaches its maximum deviation from zero. Thus, genes assigned to a GS's LE are the specific genes that account for this GS's significant enrichment or depletion signal [26].

To interpret gsea results comprehensively, GSs were mapped using network-based algorithm described previously [28]. In summary, the latter organise GSs that passed the significance threshold (FDR < 0.001) using forces of attraction, *i.e.*, edge-weights, exerted in cases where GSs share more than or equal to 5% of their member genes. Consequently, pairwise shared LE members between the GSs determined their position and organisation within the network clusters. The emerging transcriptional landscape was then visualised using the yFiles organic layout in Cytoscape 3.8.2 [29].

Real-time PCR to verify IFN-I regulated responses in whole blood

Custom array RT2 profiler PCR plates were ordered from Qiagen (Hilden, Germany), and the protocol from the manufacturer was followed using 200 ng of RNA as input with the QuantStudio5 equipment from Thermo Scientific (Waltham, MA, USA). All samples were run in doublets, and the mean of the results for the three housekeeping genes GADPH, ACTB, and HPRT1 was used as normalisation base. The $2^{-\Delta\Delta Ct}$ method was used to provide fold change values for each gene for each individual relative to the mean result of the healthy controls. Differences between groups were analysed by the Student's *t*-test in Prism (Graph Pad Software, Inc., San Diego, CA, USA), $P < 0.05$.

Analysis of autoantibodies against interferons, IL-22, and organ-specific proteins

Binding and neutralising autoantibodies against organ-specific targets, IFN-α2, IFN-α8, IFN-ω, and IL-22 were analysed by radioimmune assay (RIA) or enzyme-linked immunosorbent assay (ELISA) and antiviral interferon neutralisation assay (AVINA), respectively, as described previously [6–8,30]. The organ-specific targets for autoantibody radioimmune analysis included in Table 1 were 21-hydroxylase, 17-hydroxylase, glutamic acid decarboxylase-65, NACHT leucine-rich-repeat protein 5, aromatic L-amino acid decarboxylase, tryptophan hydroxylase, tyrosine hydroxylase, and side-chain cleavage enzyme.

Results

Global microarray RNA expression analysis reveals impairment of B cell and interferon pathways

To explore immune cell signalling pathways in the blood of APS-1 patients, we performed transcriptional studies in whole blood and compared with age- and gender-matched healthy control subjects. From the microarray results, the principal component analysis (PCA) revealed no major clustering of APS-1 nor healthy controls (Fig. S1). We observed that the individual responses varied to a high degree between individuals, and this was not affected by the different *AIRE* mutations. However, the gene enrichment analysis based on differentially expressed genes (FDR < 0.05) identified one large cluster of the IFN-I pathway to be downregulated in APS-1 patients while also B cell regulation, the clathrin/endocytic pathway, and "cellular response to pH" were significantly less active in patients compared to healthy controls. Clusters annotated "cell cycle and transcription activity," "detection of biotic stimuli," "responses to viral/bacterial exposure," and "mitochondrial activity" were found to be more activated in APS-1 patients (Fig. 1A,B, Fig. S3). Raw data from these analyses are provided online in ArrayExpress (accession E-MTAB-11630).

To dissect the downregulated pathways further, we focused on genes characteristic for the IFN-I response and B cell regulation clusters (Fig. 2A–D). Among the leading-edge (LE) genes for the IFN-I response, *MX1* (*Mycovirus resistance 1*), *Interferon-indusert transmembranprotein 3*, (*IFITM3*) and *LY6E lymphocyte antigen 6 family member E* (*Ly6E*) were significantly downregulated in APS-1. *MX1* is a prototypic IFN-I-regulated gene and a STAT1-regulated member of the dynamin-like GTPase family with a broad antiviral activity against a wide range of viruses [31], and *IFITM3* and *Ly6E* are also linked to viral immunity. Some of the characteristic IFN-I genes in the microarray experiment did not appear lower in APS-1 patients, exampled by the interferon regulatory factors (IRF) 3 and 4, where IRF3 is important for production of IFN-I and IRF4 involved in regulating inflammatory responses from myeloid cells [32]. Of specific interest is that patient 13, who is one of two informative patients without measurable antibodies against IFNα, had a consistent deviant higher expression of the whole IFN-I pathway.

Gene expression levels measured by real-time PCR experiments of genes related to the IFN I pathway did not confirm a downregulation of the IFN-I

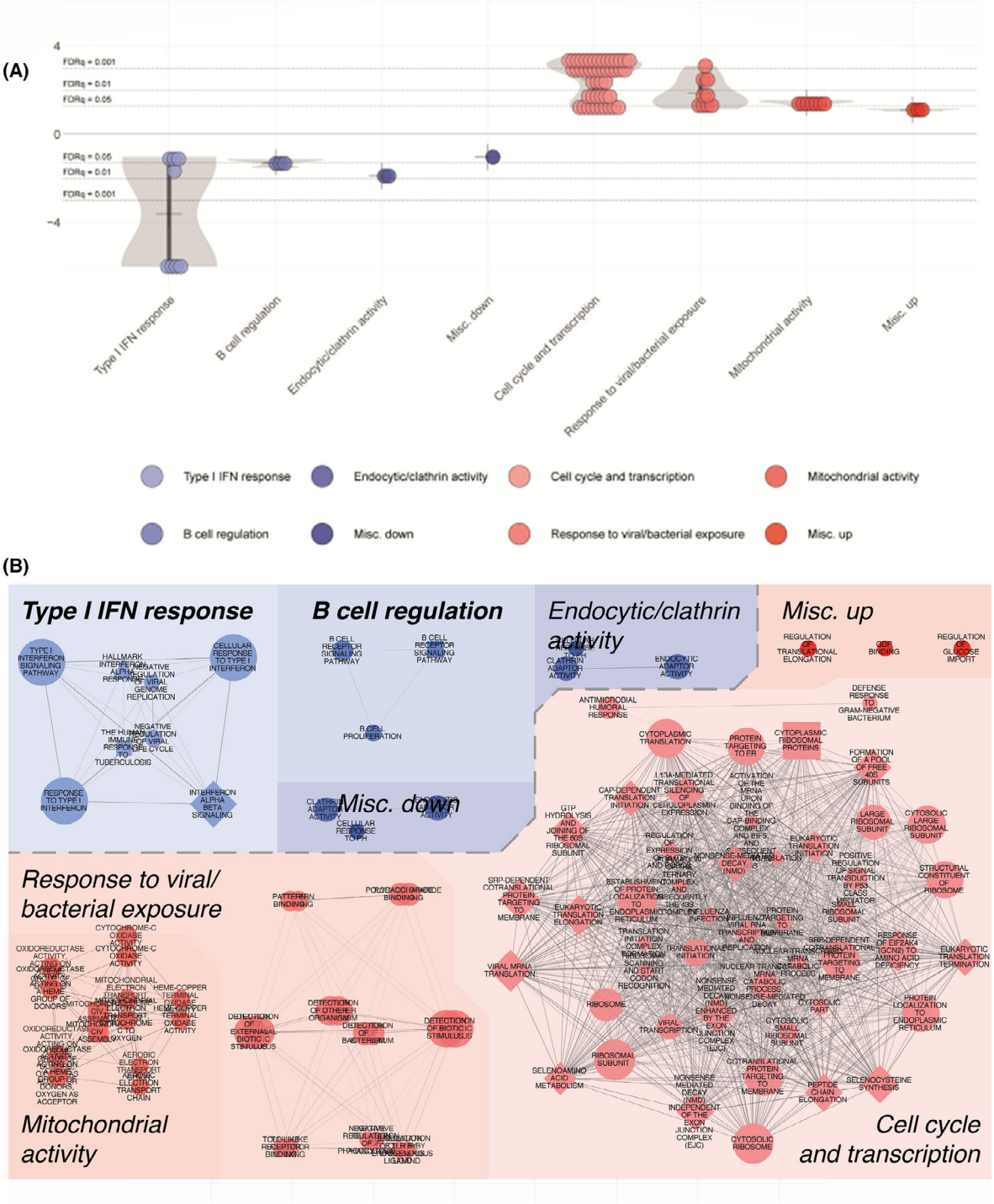

Fig. 1. Microarray expression analysis based on blood from patients with APS-1 and healthy controls. (A) Whole blood transcriptional landscape delineating all gene sets from the gene enrichment analysis of differentially expressed genes found significantly altered in APS-1 patients versus controls. Any gene set yielding a significant FDR-value of <0.1 when comparing the two groups was mapped. Network organisation and clustering reflect the underlying coordinated changes in gene expression using methodologies we described previously [28,71–73]. Node size is proportional to the significance reflected by the FDR-value. Node shape denotes the gene set's database of origin: spherical = GO, triangular = Msigdb_C2, rhomboid = Reactome, and rectangular = Wikipathways. Once interpreted and annotated, the IFN-1 response and B cell regulation clusters were deemed to be of particular interest. (B) Violin plot of directional and transformed ($\log_{10}$) FDR-values per cluster defined in B. Error bars indicate the mean ± 1 standard deviation.

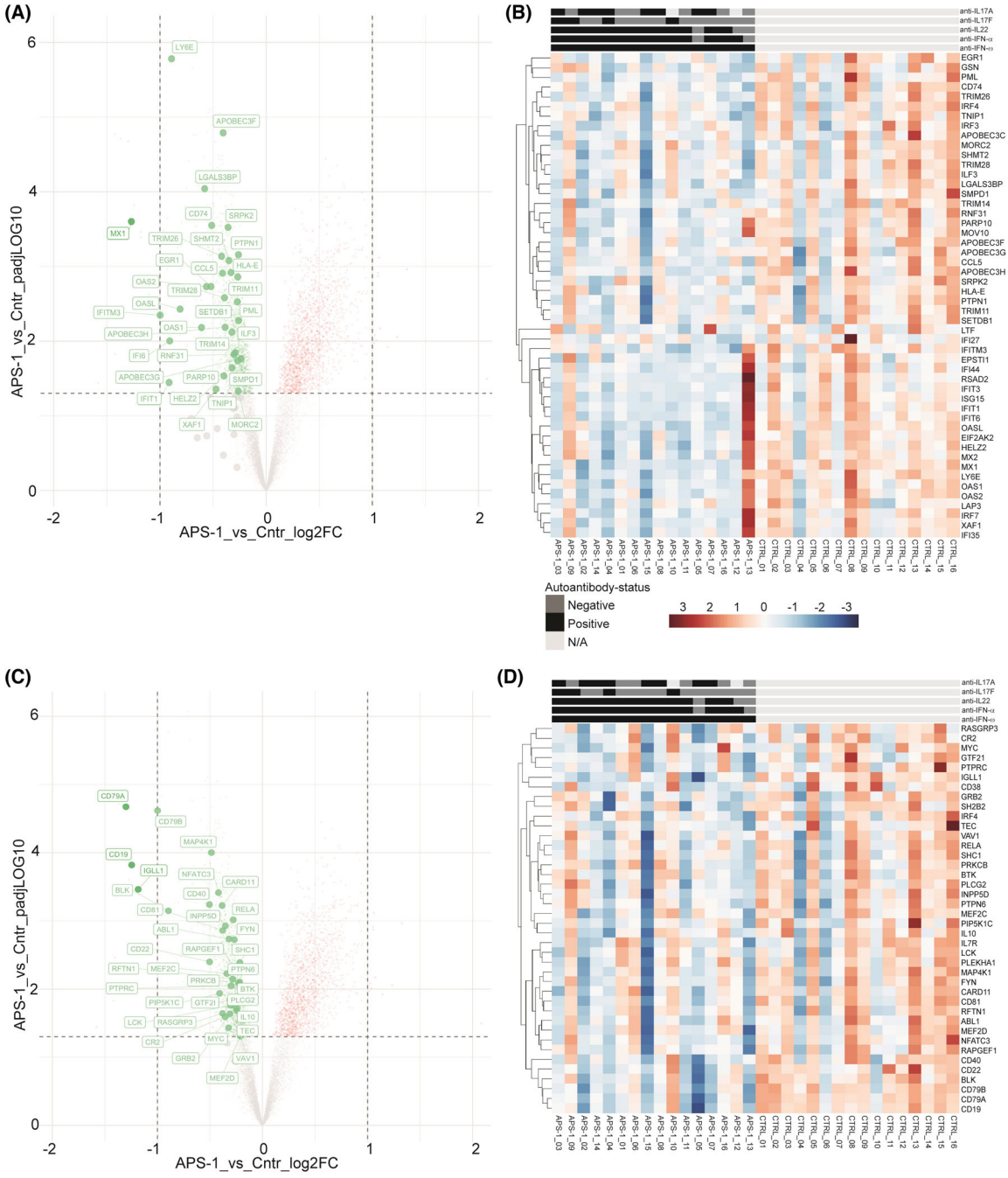

Fig. 2. Leading edge (LE) genes underlying the significant depletion of cluster I and II in APS-1 versus controls. (A) Volcano plot of the IFN-I cluster showing $\log_2$ fold change (x-axis) and $\log_{10}$ adjusted p-values (y-axis) of all genes contributing to the significant depletion signal of IFN-I response-associated genes of interest (GOI). Genes yielding an adjusted *P*-value of <0.05 were in addition labeled with their HGNC symbol. (B) Heatmap of the IFN-I cluster with rows scaled and clustered displaying the genes relevant in A and the individuals' serostatus (cytokine autoantibodies) is shown above the heat map. (C) Volcano plot of the B cell cluster indicating $\log_2$ fold change (x-axis) and $\log_{10}$ adjusted p-values (y-axis) of all genes contributing to the significant depletion signal of B cell regulation-associated genes of interest (GOI). Genes yielding an adjusted *P*-value of <0.05 were in addition labeled with their HGNC symbol. (D) Heatmap of the B cell cluster with rows scaled and clustered displaying the genes relevant in A and individuals' serostatus (cytokine autoantibodies) is shown above the heat map. For the cytokine antibody status in (B) and (D), neutralisation and binding results of autoantibodies have been merged.

pathway in APS-1 patients based on *IFI44*, *IFITM3*, *IRF3*, *CD74*, *ISG15* analysis (Fig. 3A), although upregulation of IFN-I gene expression in patient 13 was still consistently found. To be able to differentiate between IFN-I and IFN-II responses, we also performed real-time PCR on two important genes for the IFN-II responses, namely *IFNGR1 and JAK2*. These were increased in APS-1 patients compared to controls (Fig. 3B).

In the B-cell regulation cluster (Fig. 2C,D), *CD79A* and *B*, *CD19*, and *IGLL1* were all among the LE genes from the microarray experiment. Downregulation of B cell pathway genes (*CD79A*, *CD79B*, *CD83*, *JUN*, *CD19*) were also consistently observed in APS-1 patients by real-time PCR, although some of the genes failed to reach statistical significance (Fig. 3C). It is worth mentioning that *CD74*, being involved in both the IFN-I and B cell pathway (in antigen presentation), was significantly downregulated in patients in the microarray experiment, although no large deviations of expression between the groups were noted from the real-time verification.

At the individual level, heterogeneity was evident both in the control and the patient group for IFN-I and B cell responses. As all included APS-1 patients had measurable IFN-ω antibodies in their sera, and most were confirmed to be able to neutralise IFN viral responses *in vitro*, we could not determine the dependency of the IFN-I responses to IFN-I autoantibodies.

Real-time PCR of representative genes for the viral/bacterial exposure pathway (*CLEC7A* and *S100A8*) and the mitochondrial pathway (*COX7A*) copied findings from the microarray chip and showed upregulation in APS-1 patients (Fig. S2). Genes for antimicrobial activity showed both increased activity (*S100A8*) and no variation (*IL23A*).

The level and distribution of expressed RNA can be translated to profile which cells are active or inactive in a person's blood. Using this approach, we found that CD4+ central memory T cells, erythroid CD34− CD71+ cLyA− cells, granulocytes and hematopoietic stem cells, megakaryocytes, and monocytes were upregulated in APS-1 patients compared to healthy individuals. B cell populations (pro B cells, mature naïve B cells, and mature B cells switched and able to switch), in addition to basophils and eosinophils, were downregulated (Fig. 4, Fig. S4). Notably, the main producers of IFN-I, the plasmacytoid dendritic cells (pDCs), showed less profound expression, though not to statistical significance, in APS-1 patients.

Discussion

In this comprehensive transcriptional immune profiling of whole blood from a large cohort of APS-1 patients, we identified impairment of B cell signalling and a mildly decreased level of IFN-I responses compared to healthy controls. Although this has been reported previously, our data add rigour and details and include a large number of patients for this rare disease.

Human IFN-Is play pivotal roles in coordinating antiviral host defence [33] and are also associated with pathological roles in several autoimmune disorders [34–41]. In addition, IFN-α treatment can trigger autoimmune disease [42,43]. Our findings of slightly lower expression of IFN-I signature genes in APS-1 patients' whole blood is in concordance with previous analyses on peripheral blood mononuclear cells, monocyte-derived DCs, and pDCs in APS-1 patients [14,44]. It is worth to note that even though our data do not reveal large differences between patients and controls, APS-1 is still an outlier within autoimmune disorders as most other autoimmune patients show an upregulation of IFN pathways [45]. The deficient response in APS-1 is probably caused by presence of high-titre neutralising IFN-I autoantibodies present in nearly 100% of APS-1 patients, which limit systemic IFN-I activity. Importantly, one APS-1 patient *without* neutralising IFN-α2 autoantibodies and with the rare manifestations of type I diabetes and thyroid disease was found to have a "common autoimmune profile" with upregulation of at least parts of the IFN-I response (this study and [44,46,47]). We have also previously shown an inverse correlation between neutralising IFN-I autoantibodies and type I diabetes, indicating that these antibodies may limit pathological events in the pancreas [46]. Supporting the fact that IFN-I antibodies can be beneficial in specific contexts is also a recent paper by Fillatreau *et al*., showing that lupus-patients with neutralising autoantibodies against IFN-α had less disease activity compared to those without [48].

Albeit the lower IFN-I activity, frequent severe virus infections are seldom reported in APS-1 (other than rare patients in large cohorts [2]). The antibodies might still compromise host defence in some circumstances, as exemplified by the proneness for APS-1 patients to severe *COVID-19* and *Herpesvirus* infections [49–54]. Another report does however rebut this view [55], and our own observations through a study of 36 Norwegian APS-1 patients found that vaccinated individuals with IFN-I autoantibodies were protected against severe SARS-CoV2-infections (Wolff *et al*. submitted manuscript). This suggests that the presence of IFN-I autoantibodies is a moderate risk factor rather

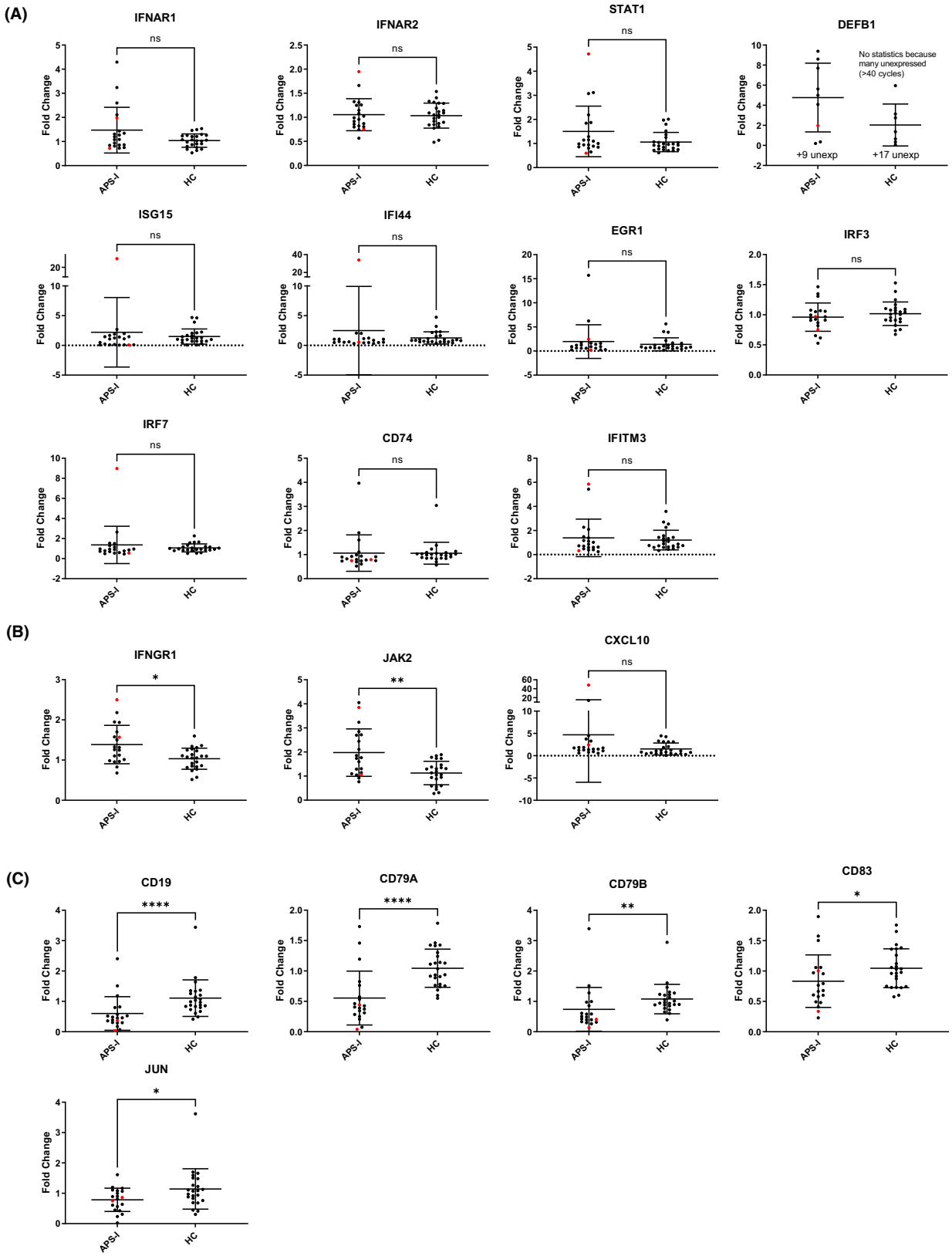

Fig. 3. Real-time PCR on designated pathways from the microarray study. (A) Expression of IFN-I regulated transcripts in whole blood from APS-I patients compared to age-and gender-matched healthy controls using quantitative PCR. (B) Expression of IFN-II regulated transcripts in whole blood from APS-I patients compared to age-and gender-matched healthy controls (CXCL10 is regulated by both IFN-Is and IFN-II). All controls and all patients with IFN-α antibodies are labeled by a black circle whereas two odd patients without IFN-α antibodies are labeled by a red circle. (C) Expression of B cell relevant transcripts in whole blood from APS-I patients compared to age-and gender-matched healthy controls using quantitative PCR. Differences between groups were analysed using the Student's *t*-test, $P < 0.01$ (although *P*-values below 0.05 was also annotated) $*P < 0.05$; $**P < 0.01$.

than a determinant for severe COVID-19. Indeed, IFNs show a high degree of redundancy and other IFNs may come into play if specific IFNs are neutralised. APS-1 patients rarely have antibodies against IFN-β with high affinity for the receptors IFNAR1 and IFNAR2 [2,56], which could compensate for deficient IFN-α/ω mechanisms. Still, the impact of circulating cytokine antibodies in APS-1-patients, and how they affect immune activity in tissues, is a mystery.

Thymic defects due to *AIRE* mutations create a severe break of T cell tolerance, but B cell tolerance is also affected since patients develop autoantibodies against a range of self-molecules [24]. Also, the mere loss of T cell tolerance directly implicates peripheral B cell mechanisms as T helper functions in these patients should be aberrated. B cells are further vital as antigen-presenting cells in the thymus and periphery and are necessary to maintain the pool of Tregs in mice [57–60]. Hence, they are crucial for sustaining peripheral tolerance [13]. The B cell-specific CD19 was one of the LE molecules, which lead to the result of deregulation of B cells in APS-1 in the microarray analysis, further supported by downregulation of CD19 in whole blood measured by real-time PCR. However, previous studies have suggested that CD19+ B cell subset levels are comparable to those in healthy subjects in immunotyping approaches [14,18,19]. It is commonly known that RNA levels do not always match protein levels, but the implications of the observed deviations are not clear. The transcriptomic

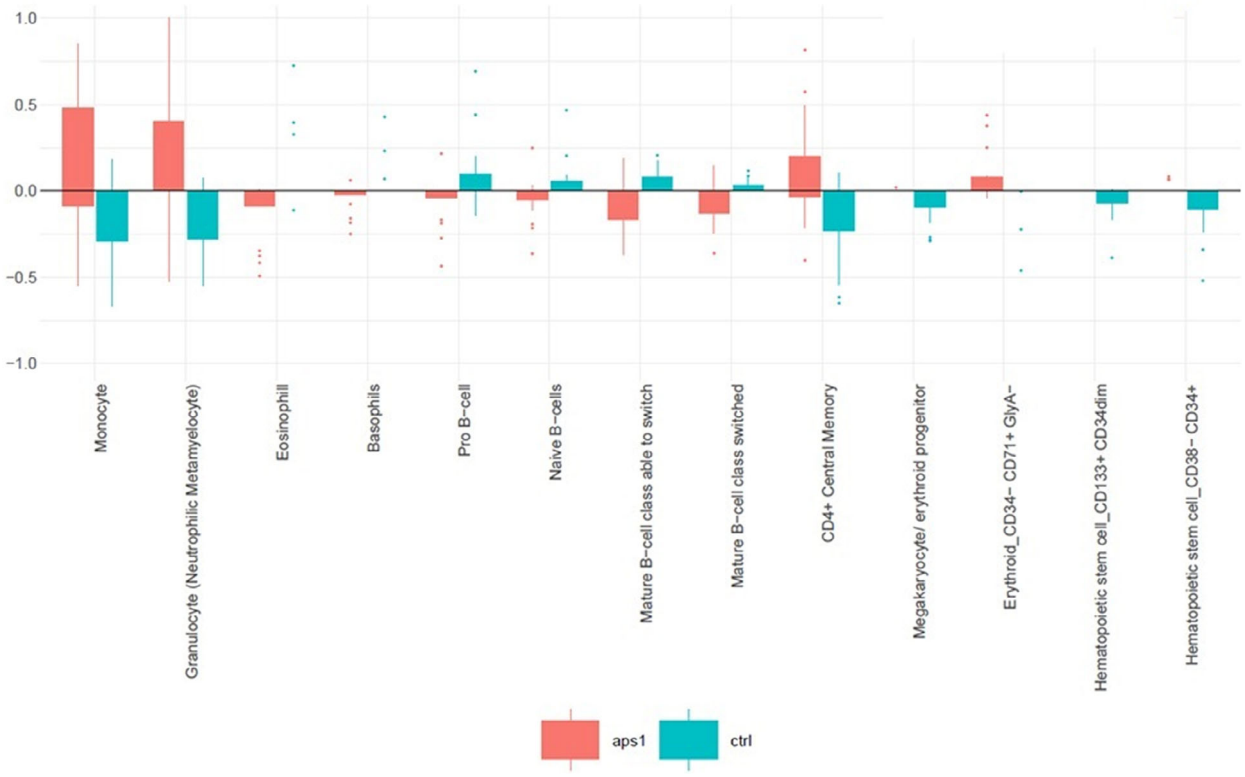

Fig. 4. Significant alterations in immune cell subsets inferred via gene signature-based expression deconvolution. Boxplot of immune cell subsets comprised in the DMAP signatures of ImmQuant [74], which showed significant differences between APS-1 patients and controls.

microarray profiling further revealed expression of the B cell associated CD79A/B, CD74, CD40, and IGLL1 to be reduced in whole blood from APS-1 patients compared to controls [61–64], supported to some extent by real-time qPCR. Hence, our data consistently showed lower B cell regulation/activity in APS-1 patients, and although the deconvolution approach suggests distinct B cell subsets to be affected, this needs to be verified by proteomic techniques. Indeed, Sng et al previously published data suggesting that early B cell development and initial tolerance are intact in APS-1 individuals and that aberrant T cells cause the later B cell deviations [13].

The genetic underlying reason for APS-1 is simple as mutations in only one gene cause disease. However, the immune functional implications of *AIRE* deficiency are extremely complex, as both autoimmune and immune deficient mechanisms come into play at the same time and contribute to the pathogenesis and immune cell disturbances. It might be possible to link lower B-cell regulation to IFN-I activity as pDCs, CD38+ CD43+ plasmablasts and early-stage transitional B cells [65–69] can produce IFN-Is in the context of autoimmunity. Also, IFN-α have been found to induce differentiation of regulatory B cells through IL10-production [69]. Perpetual IFN-I receptor stimulation can furthermore regulate B cell signalling and keep it over the "tolerance threshold," thereby boosting development of autoreactive B cells. Of relevance is also the strong connection between IFN-I and IFN-II responses. While the IFN-I responses were decreased or maintained in APS-1 subjects in our study, the IFN-II (IFN-γ) axis seemed to follow a "regular autoimmune profile" with increased responses in patients. Indeed, this fits with the findings of Break *et al.* [70], where mucosal fungal infection susceptibility in APS-1 was shown to be caused by exaggerated local IFN-II mechanisms.

There are obvious limitations to our study. The microarray analysis and the real-time PCR are both executed on a relatively large population of APS-1 patients, and the starting material is whole blood in both cases. Even though, the results did not replicate within the two methods, which could be due to differences in their RNA targeting approach and their sensitivity. The difference in the source of cells/biological material is probably also the reason why, e.g., the IFN-I profile is less disturbed found by us here in whole blood compared to our previous studies focusing on isolated mononuclear cells or even purified cell suspensions which have profound IFN-I expression profiles [44,46]. Still, when we here can mirror in whole blood previous findings regarding normalised or even decreased IFN-I-responses and decreased number of B cells in APS-I-patients, this is a strength rather than a weakness. Unfortunately, we do not have more material from matched patients taken at the same time. Studies that combine molecular immune responses in different compartments, under different conditions (e.g., after vaccine administrations, in whole blood vs. peripheral blood mononuclear cells, in isolated cell subsets etc.) and at multiple timepoints, should follow our study.

To sum up, APS-1 patients show downregulation of IFN-I and B cell signalling in blood. Further studies are needed to understand how this may impact the proteomic and cell level, and how the IFN-I autoantibodies impact on immune cell activity within tissues.

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Conflict of interests

ND owns the company he works for (2cSysBioMed, Contra, Switzerland). The company was paid to perform some of the bioinformatical analyses in the article. The other authors have no relevant financial or non-financial interests to disclose.

Author contributions

BEO, ESH and ASBW conceived and designed the study; ESH recruited patients; BEO, ND, DD, AM, ESH and ASBW acquired, processed, analysed, and interpreted the data. BEO and ASBW drafted the manuscript. All authors critically revised the manuscript for important intellectual content.

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Peer review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/1873-3468.14625.

Data availability statement

The data that support the findings from the microarray approach of this study are openly available in ArrayExpress at ArrayExpress < EMBL-EBI, reference number E-MTAB-11630.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Principal component analysis.

Fig. S2. Real-time PCR on designated pathways from the microarray study for clusters III-VII (not the IFN and B cell regulation clusters).

Fig. S3. Volcano plots of the leading edge (LE) genes underlying the significant depletion/up-regulation of clusters III-VII in APS-1 patients versus controls in the microarray array.

Fig. S4. All alterations in immune cell subsets inferred via gene signature-based expression deconvolution.