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Suppressed immune profile in children with combined type 1 diabetes and celiac disease

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Summary

Children diagnosed with a combination of type 1 diabetes (T1D) and celiac disease (CD) show a dysregulated T helper type 1 (Th1)/Th17 response. Besides the cellular involvement, several soluble immune markers are involved in the autoimmune process of both T1D and CD. Only few studies have examined the peripheral pattern of different cytokines, chemokines and acute-phase proteins (APP) in children with combined T1D and CD. To our knowledge, no studies have evaluated the serum levels of adipocytokines and matrix metalloproteinases (MMPs) in this context. The purpose of the present study was to acquire more knowledge and to gain deeper understanding regarding the peripheral immunoregulatory milieu in children with both T1D and CD. The study included children diagnosed with both T1D and CD (n = 18), children with T1D (n = 27) or CD (n = 16) and reference children (n = 42). Sera were collected and analysis of 28 immune markers (cytokines, chemokines, APPs, adipocytokines and MMPs) was performed using the Luminex technique. The major findings showed that children with a double diagnosis had lower serum levels of interleukin (IL)-22, monocyte chemoattractant protein (MIP)-1α, monocyte chemoattractant protein (MCP)-1, procalcitonin, fibrinogen, visfatin and matrix metalloproteinase (MMP)-2. These results indicate a suppressed immune profile in children with combined T1D and CD, including Th17 cytokines, chemokines, APPs, adipocytokines and MMPs. We conclude that, besides cytokines and chemokines, other immune markers, e.g. APPs, adipocytokines and MMPs, are of importance for further investigations to elucidate the heterogeneous immune processes present in patients diagnosed with T1D in combination with CD.

Keywords: celiac disease, children, immune markers, type 1 diabetes

Introduction

Type 1 diabetes (T1D) and celiac disease (CD) are complex T cell-driven-mediated autoimmune diseases, including several components of both the innate and adaptive immune system [1–3]. Children diagnosed with a combination of T1D and CD show a diminished T helper type 1 (Th1)-like profile [4], and recent studies also suggest a dysregulated Th17 response [5,6]. As well as the cellular involvement, several soluble immune markers are involved in the autoimmune processes seen in both T1D and CD. Only a few studies have examined the peripheral pattern of different cytokines, chemokines and acute-phase proteins (APPs) in children with a combined diagnosis of T1D and CD. Further, no studies have, to our knowledge, evaluated the serum levels of adipocytokines and matrix metalloproteinases (MMPs) in this context.

The autoimmune process in T1D is associated with both cell-mediated immunity, including autoreactive $CD4^+$ and $CD8^+$ T lymphocytes, and humoral immunity including autoantibodies [3]. The effector function of the interferon (IFN)-γ-producing Th1 cells has a key role in the destruction of the insulin-producing β cells, and evidence suggests that Th17 lymphocytes also play an important role [3,5,7]. Recently, an increased Th22

response as well as increased interleukin (IL)-22 levels in patients with T1D have been reported [6,8]. Studies of peripheral immune markers in patients with T1D have shown alterations in the expression of several immune markers, e.g. IL-1α, IL-1β, IL-2, IL-6, IL-10, tumor necrosis factor (TNF)-α/TNF-β, IFN-γ, macrophage inflammatory proteins (MIP)-1α, IL-1β and MMPs [9–13].

In celiac disease, the activated $CD4^+$ T lymphocytes secrete high levels of proinflammatory cytokines (IFN-γ, IL-1β, TNF-α, IL-6 and IL-8), inducing Th1 helper cells to secrete large amounts of IFN-γ [14–16] and Th2 helper cells to produce Th2 cytokines (IL-4, IL-10), thus affecting both the cell-mediated immunity and proinflammatory responses and the humoral immune immunity and downregulation of the inflammatory processes [14,15,17]. The secreted proinflammatory cytokines promote tissue damage due to inflammation and also induce the secretion of MMPs which contribute to tissue remodeling leading to villous atrophy [18,19]. Several studies have shown that besides Th1 and Th2 cytokines, proinflammatory Th17 lymphocytes secreting IL-17A, IL-21, IL-22 and IL-33 are also involved in the pathogenesis of CD [20,21].

APPs are markers of innate immunity and are frequently associated with inflammatory status, but the relationship between low-grade inflammation and autoimmune diseases is not well studied. Several acute-phase inflammatory markers have been reported to be increased in both T1D and CD [22,23]. The T cell-driven inflammation leads to secretion of proinflammatory TNF-α and IL-1β, which contribute to the inflammation of β cells in T1D, as well as inducing histological changes in the small intestinal mucosa in CD [24,25]. Secreted TNF-α and IL-1β induce synthesis of IL-6 and activate hepatocytic receptors to synthesize different APPs, e.g. procalcitonin (PTC), ferritin, fibrinogen, serum amyloid A (SAA) and tissue plasminogen activator (tPA). Procalcitonin, stimulated by IL-6 and TNF-α, act as a monocyte chemoattractant in inflammatory processes [26]. Immunological processes seen in different autoimmune diseases also have an impact on ferritin synthesis through TNF-α and IL-1α secreted by activated macrophages [27,28]. Fibrinogen can stimulate macrophages to secrete several chemokines and cytokines, such as monocyte chemoattractant protein (MCP)-1, MIP-1α, MIP-1β, IL-6, IL-8, TNF-α and matrix metalloproteinase (MMP)-1 [29,30]. SAA influences the innate immune responses in autoimmune diseases by stimulating neutrophil granulocytes and monocytes to release TNF-α [31]. tPA has been shown to modulate macrophages, $CD4^+$ and $CD8^+$ cells, and can induce the expression of proinflammatory chemokines in inflammatory diseases, thereby having a significant role in tissue destruction and tissue remodeling [32,33].

Studies have shown that adipocytokines, e.g. visfatin and resistin, have characteristics of classical cytokines with important proinflammatory, regulatory and immunomodulating properties, thereby influencing several autoimmune diseases [34–36]. Visfatin plays a substantial role in a wide range of chronic inflammatory diseases and immune responses by the ability to stimulate both pro- and anti-inflammatory cytokines such as IL-1β, IL-1Ra, IL-6, IL-10 and TNF-α [34,37]. The regulatory role of resistin in different inflammatory disorders is mediated by the capacity to stimulate proinflammatory cytokines such as TNF-α, IL-1β and IL-6 [35,36,38].

MMPs are important endopeptidases involved in tissue remodeling, through their ability to degrade extracellular matrix in both physiological and pathological conditions [39,40]. MMP-1, MMP-2 and MMP-3 can regulate TNF-α, which plays an important pathogenic role in both T1D and CD [25,40,41]. Depending on the context, MMPs can promote or inhibit the inflammatory response by the direct proteolytic effect on cytokines and chemokines [39,40,42]. Dysregulation of MMPs has been observed in both T1D [43,44] and CD [21,45].

Taken together, the peripheral immunoregulatory milieu is not well studied in children diagnosed with combined T1D and CD. Thus, the purpose of the present study was to acquire more knowledge and to gain deeper understanding of the peripheral blood immunoregulatory milieu in children with combined T1D and CD.

Material and methods

Participants

The study included four study groups: children diagnosed with both T1D and CD (n = 18), children diagnosed with T1D (n = 27) or CD (n = 16) and a control group (reference children, n = 42). The participants in the different groups were age- and gender-matched as far as possible. General criteria for participating in the study was that the children in all study groups should not show any signs of allergy, colds or other infections at time of sample collection.

The control group consisted of healthy children, and neither these reference children nor their first-degree relatives displayed any clinical signs of T1D, CD or other autoimmune diseases.

Type 1 diabetes was diagnosed according to the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines [46], including symptoms of diabetes plus casual plasma glucose concentration ≥ 11·1 mmol/l (200 mg/dl), fasting plasma glucose ≥ 7·0 mmol/l (≥ 126 mg/dl) or 2-h post-load glucose ≥ 11·1 mmol/l (≥ 200 mg/dl) during an oral glucose tolerance

test (OGTT). Duration of T1D was defined from the date of diagnosis. The median duration of disease was 4·2 years (range = 0·3–12·9) in the single-diagnosis group and 3·5 years (0·1–10·8) in the double-diagnosis group.

Celiac disease was diagnosed according to the modified version of the European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) criteria [47]. Duration of CD was defined as the date of biopsy-confirmed diagnosis and the median duration of disease was 5·9 years (range = 0·6–11·7) in the single-diagnosis group and 2·1 years (0·4–10.2) in the double-diagnosis group. All children diagnosed with CD were on a gluten-free diet for at least 3 months after diagnosis before inclusion in the study.

All children diagnosed with T1D and/or CD were tested for presence of anti-tissue transglutaminase (tTG) immunoglobulin (Ig)A. Serum anti-tTG IgA was measured by a fully automated EliA™ Celikey® IgA assay (Pharmacia Diagnostics, Freiburg, Germany), according to the manufacturer's instructions. Anti-tTG IgA values > 10 U/ml were considered positive.

One hundred per cent within the T1D group, 81% within the CD group (13 of 16) and 67% in the double-diagnosis group (12 of 18) were anti-tTG IgA-negative.

The participants in the double-diagnosis group, with the exception of one girl and three boys, were diagnosed with T1D before diagnoses of CD.

Sample collection

Blood samples were collected in Vacutainer tubes without anti-coagulant (BD Biosciences, San Jose, CA, USA), at the Linköping University Hospital, Linköping, the Ryhov County Hospital, Jönköping and School Health Services in Linköping. To minimize seasonal variation, blood sampling of all study groups was performed during the whole year. More samples were, however, collected during the spring and conversely, fewer samples during the summer period compared to the other seasons, due to the vacation period during summertime (Table 1).

Thirty min after sampling, sera were separated by centrifugation of the whole blood samples at 2000 ***g*** for 10 min. Thereafter the sera were aliquoted and stored at −80°C until analysis. Multiple freeze–thaw cycles were avoided, because this is detrimental to many soluble immune markers in sera.

Analysis of immune markers

Twenty-eight immune markers in blood sera were analyzed by using multiplex fluorochrome sandwich immunoassays based on Luminex™ xMAP technology (Luminex; Bio-Rad Laboratories, Hercules, CA, USA) Bio-Plex assays (Bio-Rad Laboratories) on the instrument Bio-Plex 200 system (Bio-Rad Laboratories), according to the manufacturer's instructions. Median fluorescence intensity (MFI) for each sample was registered and analyzed with Bio-Plex Manager™ Software version 5.0 (Bio-Rad Laboratories). The analyte concentrations were estimated using a five-parameter logistic model standard curve.

The cut-off values for minimum detectable concentrations for each immune marker are presented in Supporting information, Table S1.

Quality controls (recombinant protein supplied by the manufacturer) for each immune marker were included in each experiment to control for the assay performance. All quality controls were within the expected range (determined by the manufacturer). To evaluate the reproducibility of the assay, intra- and interassay coefficient of variation (CV, presented as percentage of SD) were calculated for the quality controls assayed in duplicate. Recommended intra- and interassay variations for Luminex assays should be < 10% for intra-assay and < 20% for interassay [48]. The intra- and interassay variabilities in our analysis were 6·0 and 6·2%, respectively. Reproducibility of the data was evaluated by estimation of intra- and interassay variation for two immune markers, which were analyzed in all samples of the cohort. The interassay CV was 1·3% for IL-6 and 2·7% for IL-10.

Cluster analysis

For exploratory data analysis, hierarchical cluster analysis (HCA) was performed to identify similarities/differences

Table 1. Distribution of sample collection over the seasons in the different study groups: children with combined type 1 diabetes and celiac disease (T1D + CD), children with T1D, children with CD and reference children (control)

	Whole study cohort *n* = 103	T1D + CD *n* = 18	T1D *n* = 27	CD *n* = 16	Control *n* = 42
Season of the sample collection	*n* (G/B)	*n* (G/B)	*n* (G/B)	*n* (G/B)	*n* (G/B)
Winter (December, January, February)	21 (9/12)	3 (0/3)	4 (3/1)	9 (5/4)	5 (1/4)
Spring (March, April, May)	54 (29/23/2[a])	6 (4/1/1[a])	13 (7/6)	6 (4/2)	29 (14/14/1[a])
Summer (June, July, August)	8 (1/4/3[a])	1 (0/1)	4 (1/3)	0	3[a]
Autumn (September, October, November)	20 (11/8/1[a])	8 (3/4/1[a])	6 (3/3)	1 (1/0)	5 (4/1)

G = girls; B = boys.

[a]Gender data not available.

[49] in immune profiles between children with a double diagnosis, children with a single diagnosis and children without these diagnoses (i.e. reference children), respectively. To find relatively homogeneous clusters, a complete linkage method was used. Thus, a dendrogram of the study population based both on the immune marker expression profiles and the diagnosis was generated. The cluster analysis was performed using software package Hierarchical Clustering Explorer version 3.5 (Human–Computer Interaction Laboratory, University of Maryland, College Park, MD, USA).

Statistics

Differences between the different study groups were examined in the whole cohort as well as in the clustered groups. As the levels of immune markers were not normally distributed, Mann–Whitney *U*-test was used for comparison of the different diagnosis groups within the whole study cohort and within the different clusters. A probability level of $< 0{\cdot}05$ was considered statistically significant.

Spearman's correlation coefficients were calculated to determine the relationship between the analyzed immune marker levels in each study group. Spearman's coefficients $r > 0{\cdot}50$ and $P < 0{\cdot}01$ were considered statistically significant. All statistical analyses were performed using Statistical Packages for the Social Sciences (SPSS) version 25.0 and GraphPad Prism version 6.0 for Windows.

Ethics

This study was approved by the Research Ethics Committee of the Faculty of Health Sciences, Linköping University, Linköping, Sweden and the Regional Ethics Committee for Human Research, Linköping (approval number: Dr M89-2006 and complementary Dr: 2012/27-32). Information was given both orally and written to all participants and their parents or responsible guardians. All children received information adapted for their age. Informed consent was obtained from the children's guardian.

Results

Twenty-eight immune markers were measured in sera from children diagnosed with T1D and/or CD as well as from reference children. Differences between the study groups were examined in the whole cohort. To identify possible specific patterns of immune markers between the study groups, unsupervised HCA was performed. The complete linkage clustering generated a dendrogram showing levels of immune markers according to the participants' diagnoses. Using a cut-off value of 0·5 in the hierarchical cluster analysis, three clusters with similar immune profiles were identified (clusters A–C, Fig. 1). However, the dendrogram showed an asymmetric distribution of immune markers between the different study groups. Characteristics

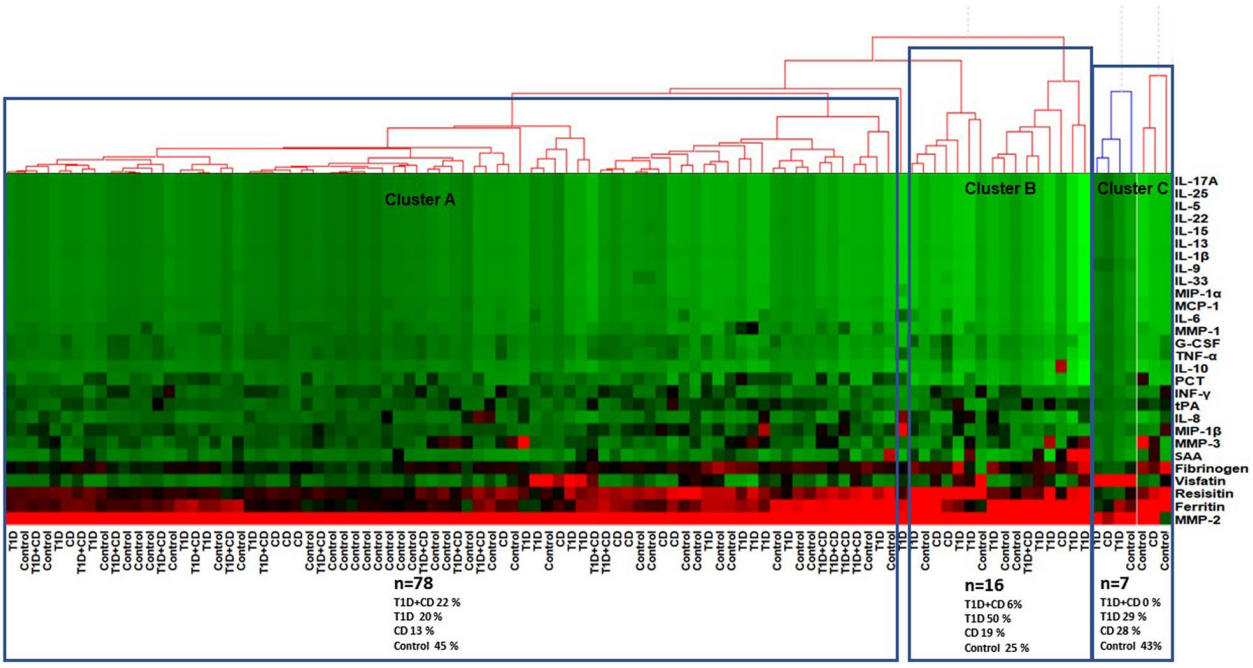

Fig. 1. Hierarchical clustering dendrogram of all 28 measured immune markers in children with combined type 1 diabetes and celiac disease (T1D + CD, $n = 18$), T1D ($n = 27$), CD ($n = 16$) and reference children (control, $n = 42$). The dendrogram shows the clustering of participants according to the immune marker profile and diagnosis. The heat-map indicates the levels of immune markers for each child.

of the different diagnosis groups, representing the whole study cohort as well as the different clusters, are presented in Table 2. Each cluster (A–C) included children with T1D, children with CD and reference children. Interestingly, children diagnosed with both T1D and CD were represented only in clusters A and B. The largest cluster (cluster A) included 75% of the participants and the distribution of diagnoses in this cluster is very similar to the distribution in the whole study cohort (Table 2, Fig. 1). Cluster B, including 15·5% of the participants, consisted predominantly of children with T1D (50%) but only 6% of children with a combined T1D and CD diagnosis. The smallest cluster (cluster C) included only 6·5% of the participants and was consisted predominantly of children with a single diagnosis (T1D = 29% and CD = 28%).

Regarding distribution of participants in the different clusters and different study groups, the median age of participants and duration of T1D and/or CD in cluster A, the largest cluster, showed most similarities with the whole study cohort, including all participants tested positive for anti-tTG-IgA.

To identify differences in levels of immune markers within the clusters and between the children with T1D and/or CD, the two larger clusters (clusters A and B), which included all diagnosis groups, were compared. The results showed alterations in levels of some immune markers between the diagnosis and reference group within the whole cohort as well as in the different clusters (Fig. 2). Serum levels of the immune markers (cytokines and chemokines, APPs, adipocytokines and MMPs) in children with different diagnoses, detected in the whole study cohort as well as in cluster A, respectively, are presented in Tables 3 and 4.

In the whole study cohort, there were no differences between the diagnosis groups regarding serum levels of IFN-γ and Th17 cytokines (IL-17A, IL-22, IL-25, IL-33).

Table 2. Characteristics of the study groups (whole study cohort and clusters A–C); children with combined type 1 diabetes and celiac disease (T1D + CD), children with T1D, children with CD and reference children (control)

		Whole study cohort	Cluster A	Cluster B	Cluster C
	n	103 (100%)	78 (76%)	16 (15·5%)	7 (6·5 %)
T1D + CD	*n*	18 (17%)	17 (22%)	1 (6%)	0 (0 %)
	Gender, G (*n*)/B (*n*)	7 / 9 (2[a])	6 / 9 (2[a])	1 / 0	–
	Age (years)	11·5 (7·0–15·4)	12·0 (7·0–15·5)	8·0 (8·0–8·0)	–
	Girls	9·5 (7·5–13·2)	8·8 (7·5–13·0)	8·0 (8·0–8·0)	–
	Boys	12·0 (7·0–15·4)	12·0 (7·0–15·5)		–
	Duration T1D (years)	3·5 (0·1–10·8)	3·4 (0·1–10·8)	4·5 (4·5–4·5)	–
	Girls	2·6 (0·1–5·5)	2·5 (0·1–5·5)	4·5 (4·5–4·5)	–
	Boys	3·6 (1·2–10·8)	3·6 (1·2–10·8)		–
	Duration CD (years)	2·1 (0·4–10·2)	1·9 (0·4–10·2)	4·6 (4·6–4·6)	–
	Girls	4·5 (0·5–10·2)	3·2 (0·5–10·2)	4·6 (4·6–4·6)	–
	Boys	1·9 (0·4–4·2)	1·9 (0·4–4·2)		–
T1D	*n*	27 (26%)	16 (20%)	8 (50%)	2 (29 %)
	Gender G (*n*)/B (*n*)	14/13	9/7	4/4	1/1
	Age (years)	13·0 (6·0–17·4)	12·8 (6·0–17·0)	17·0 (8·0–18·0)	12·5 (11·0–14·0)
	Girls	12·7 (8·4–17·2)	12·5 (10·0–14·0)	15·0 (8·0–18·0)	11 (11·0–11·0)
	Boys	13·5 (6·0–17·4)	13·0 (6·0–17·0)	17·0 (14·0–17·0)	14 (14·0–14·0)
	Duration T1D (years)	4·2 (0·3–12·9)	3·0 (0·3–10·2)	5·5 (3·3–12·9)	6·7 (6·6–6·7)
	Girls	5·7 (0·8–12·0)	4·2 (0·8–10·2)	6·0 (3·8–12·0)	6·6 (6·6–6·6)
	Boys	3·3 (0·3–12·9)	1·8 (0·3–5·5)	4·5 (3·3–12·9)	6·7 (6·7–6·7)
CD	*n*	16 (15%)	10 (13%)	3 (19%)	2 (28 %)
	Gender G (*n*)/B (*n*)	10/6	5/5	2/1	2/0
	Age (years)	10·0 (7·4–17·3)	10·0 (7·0–14·0)	14·5 (10·0–17·0)	15 (13·0–17·0)
	Girls	11·5 (7·5–17·3)	10·0 (10·0–14·0)	15·8 (14·5–17·0)	15 (13·0–17·0)
	Boys	10·0 (7·4–10·5)	10·0 (7·0–10·5)	10·0 (10·0–10·0)	
	Duration CD (years)	5·9 (0·6–11·7)	5·0 (0·6–11·0)	1·4 (0·8–11·7)	6·1 (6·1–6·1)
	Girls	6·6 (0·7–17·7)	7·1 (0·7–11·0)	6·6 (1·4–11·7)	6·1 (6·1–6·1)
	Boys	1·0 (0·6–5·8)	1·0 (0·6–5·8)	0·8 (0·8–0·8)	–
Control	*n*	42 (40%)	35 (45%)	4 (25%)	3 (43 %)
	Gender G (*n*)/B (*n*)	19/19 (4[a])	15/16 (4[a])	1/3	1/2
	Age (years)	12·0 (6·8–16·8)	11·5 (7·0–17·0)	7·8 (7·0–15·0)	14·5 (12·0–16·0)
	Girls	12·0 (8·5–16·8)	11·0 (9·0–17·0)	7·5 (7·5–7·5)	12·3 (12·0–14·5)
	Boys	12·0 (6·8–16·8)	12·0 (7·0–14·0)	8·0 (7·0–15·0)	16·0 (16·0–16·0)

Age and diabetes duration presented as median (minimum, maximum). G = girls; B = boys.

[a]Age and gender data not available.

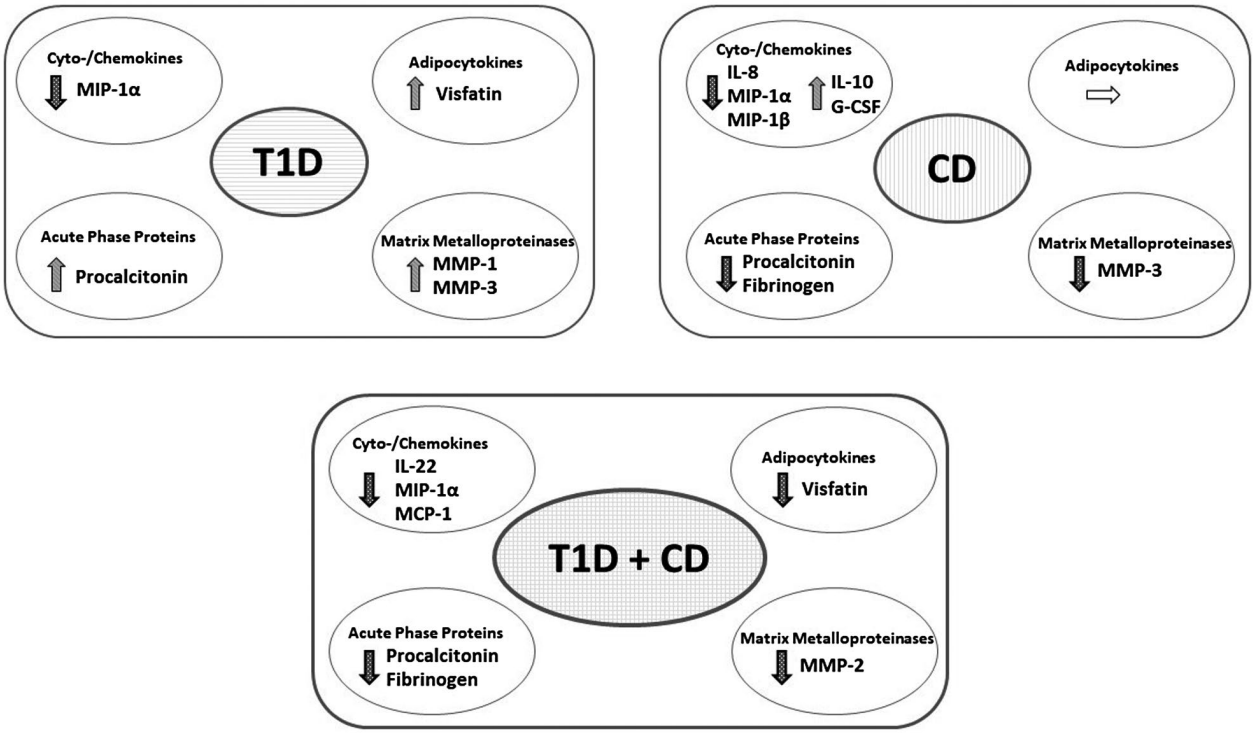

Fig. 2. Schematic overview of the most striking immune markers studied in children with type 1 diabetes (T1D), celiac disease (CD) and T1D + CD.

Levels of MMP-1 and MMP-3 and visfatin are higher in children with type 1 diabetes

The levels of procalcitonin were higher in T1D children compared to children with CD in the whole study cohort ($P < 0{\cdot}05$, Fig. 3a), as well as in cluster A ($P < 0{\cdot}05$, Fig. 3b). Type 1 diabetic children secreted higher levels of visfatin than children without this diagnosis ($P < 0{\cdot}05$, Fig. 4a) and visfatin was also correlated to duration only in children diagnosed exclusively with T1D ($r = 0{\cdot}501$, $P < 0{\cdot}01$, Fig. 4b). Secretion of MMP-1 ($P < 0{\cdot}05$, Fig. 4c) and MMP-3 ($P < 0{\cdot}05$, Fig. 4d) were higher in children with T1D compared to children diagnosed with CD. In contrast, serum levels of MMP-2 were lower in children with either T1D or CD compared to the control group ($P < 0{\cdot}01$, Fig. 4e,f). Type 1 diabetic children within cluster A had also lower levels of MIP-1α ($P < 0{\cdot}05$, Fig. 5a) compared with the control group. Thus, children diagnosed exclusively with T1D had a high secretion of procalcitonin, visfatin as well as MMP-1 and MMP-3, in contrast to a low secretion of MIP-1α (summarized in Fig. 2).

Low levels of MIP-1α but high levels of IL-10 in children with celiac disease

Children with CD had lower levels of MIP-1α compared with T1D children ($P< 0{\cdot}0001$, Fig 5a), children with combined T1D and CD ($P < 0{\cdot}01$, Fig. 5a) and also compared with the control group ($P < 0{\cdot}0001$, Fig. 5a). Children with CD also have lower levels of MIP-1α ($P < 0{\cdot}05$, Fig. 5a,b), MIP-1β ($P < 0{\cdot}05$) and IL-8 ($P < 0{\cdot}05$) compared to children in the control group, observed both within the whole study cohort as well as in cluster A. In contrast, the secretion of IL-10 was higher in children with CD compared with groups comprised of children diagnosed exclusively with T1D ($P = 0{\cdot}001$), children diagnosed with both T1D and CD ($P = 0{\cdot}001$) or children without diagnosis of either T1D or CD ($P < 0{\cdot}05$). The serum levels of granulocyte–colony-stimulating factor (G-CSF) among children diagnosed exclusively with CD was higher compared with children with a double diagnosis or no diagnosis at all ($P < 0{\cdot}05$). Children with celiac disease showed a lower secretion of several cytokines and chemokines, e.g. IL-8, MIP-1α and MIP-1β, procalcitonin, fibrinogen and MMP-3 in contrast to high secretion of IL-10 (summarized in Fig. 2).

Diminished levels of several immune markers in children with combined type 1 diabetes and celiac disease

Children with both T1D and CD had lower levels of MIP-1α ($P < 0{\cdot}05$, Fig. 5a,b) and MCP-1 ($P < 0{\cdot}05$, Fig. 5c,d) compared to children without diagnosis, observed both within the whole study cohort as well as in cluster A. The levels of procalcitonin were lower in children with

Table 3. The serum level of cytokines and chemokines in children with combined type 1 diabetes and celiac disease (T1D + CD), T1D, CD and reference children (control). Results are presented as median (minimum, maximum)

	Immune markers (pg/ml)	T1D + CD		T1D		CD		Control	
		Whole study cohort (18)	Cluster A (17)	Whole study cohort (27)	Cluster A (16)	Whole study cohort (16)	Cluster A (10)	Whole study cohort (42)	Cluster A (35)
Cytokines and chemokines	IFN-γ	824·2 (376·0–2110·3)	815·5 (376·0–2110·3)	1042·7 (116·6–1919·4)	858·2 (116·6–1919·4)	1092·6 (39·8–2514·5)	1092·6 (39·8–1822·7)	948·9 (211·1–3944·3)	919·7 (211·1–3944·3)
	IL-5	2·0 (0·0–5·7)	2·5 (0·0–5·7)	1·5 (0·0–19·6)	1·5 (0·0–10·3)	2·0 (0·0–19·0)	2·0 (0·0–7·8)	0·3 (0·0–26·3)	0·3 (0·0–26·3)
	IL-9	0·9 (0·0–26·6)	1·0 (0·0–26·6)	1·5 (0·0–1191·7)	2·1 (0·0–49·4)	0·4 (0·0–2341·7)	0·3 (0·0–122·7)	0·0 (0·0–356·7)	0·2 (0·0–27·9)
	IL-10	73·4 (0·0–132·8)	74·1 (0·0–132·8)	60·3 (0·0–434·0)	55·7 (0·0–132·2)	93·0 ↑ *1, *2, *3 (21·9–13 555·9)	82·5 (21·9–204·2)	76·6 (11·2–484·3)	80·4 (11·2–484·3)
	IL-13	1·6 (0·6–110·8)	1·8 (0·6–110·8)	5·4 (0·4–26·6)	6·0 (0·4–24·1)	1·9 (0·6–16·7)	2·0 (0·6–6·0)	2·4 (0·6–17·4)	2·5 (0·6–15·4)
	IL-17A	0·0 (0·0–0·0)	0·0 (0·0–0·0)	0·0 (0·0–7·3)	0·0 (0·0–2·2)	0·0 (0·0–24·8)	0·0 (0·0–4·4)	0·0 (0·0–1·8)	0·0 (0·0–1·8)
	IL-22	0·0 (0·0–5·4)	0·0 ↓ *1 (0·0–5·4)	0·0 (0·0–92·8)	1·5 (0·0–7·8)	0·0 (0·0–78·5)	0·0 (0·0–78·5)	0·0 (0·0–29·5)	0·0 (0·0–29·5)
	IL-25	0·0 (0·0–1·7)	0·0 (0·0–1·7)	0·0 (0·0–3·8)	0·0 (0·0–3·8)	0·3 (0·0–3·3)	0·3 (0·0–3·3)	0·0 (0·0–12·4)	0·0 (0·0–12·4)
	IL-33	0·0 (0·0–16·4)	0·0 (0·0–3·0)	0·0 (0·0–138·1)	0·0 (0·0–104·7)	0·0 (0·0–134·9)	0·0 (0·0–28·3)	0·0 (0·0–460·3)	0·0 (0·0–460·3)
	IL-1β	30·0 (10·7–63·1)	34·9 (10·7–63·1)	34·1 (4·1–116·4)	24·9 (4·1–53·2)	38·5 (10·7–64·4)	37·4 (12·9–64·4)	32·0 (11·2–131·8)	31·4 (11·2–131·8)
	IL-6	0·0 (0·0–212·8)	0·0 (0·0–212·8)	0·0 (0·0–2140·4)	0·0 (0·0–701·5)	29·5 (0·0–1046·3)	6·4 (0·0–68·7)	0·1 (0·0–528·6)	4·8 (0·0–528·6)
	IL-15	0·0 (0·0–36·8)	0·0 (0·0–25·6)	0·0 (0·0–141·0)	0·0 (0·0–12·0)	0·0 (0·0–36·8)	0·0 (0·0–36·8)	0·0 (0·0–36·4)	0·0 (0·0–141·0)
	TNF-α	389·3 (100·4–716·6)	391·3 (100·4–716·6)	340·5 (25·3–2262·0)	267·6 (25·3–782·2)	417·6 (137·8–911·2)	417·6 (146·9–713·1)	379·7 (119·3–713·1)	370·0 (119·3–713·1)
	IL-8	381·5 (139·1–4972·8)	310·7 (139·1–4972·8)	572·5 (98·8–7896·9)	250·4 (98·8–3718·8)	299·4 ↓ *3 (157·7–655·1)	265·1 ↓ *3 (196·7–655·1)	615·9 (185·5–9815·4)	618·9 1 (200·9–9815·4)
	MCP-1	66·8 ↓ *3 (48·0–138·6)	68·2 ↓ *3 (48·0–138·6)	76·3 (19·0–221·0)	84·8 (24·7–129·0)	68·6 ↓ *3 (37·4–126·5)	73·1 (38·6–126·5)	96·9 (10·6–346·4)	99·0 (21·9–346·4)
	MIP-1α	2·1 ↓ *2, *3 (0·6–62·2)	2·3 ↓ *3 (0·6–62·2)	2·5 (0·6–772·0)	2·3 ↓ *3 (1·1–144·4)	1·1 ↓ *1, *3 (0·5–3·4)	1·4 ↓ *1, *3 (0·7–3·4)	5·1 (0·8–64·7)	6·9 (0·8–64·7)
	MIP-1β	1039·1 (534·2–3042·7)	1037·5 (534·2–3042·7)	1109·5 (567·2–21142·3)	1069·4 (567·2–7616·2)	893·0 ↓ *3 (547·9–1693·6)	866·9 ↓ *3 (547·9–1693·6)	1341·7 (540·5–3979·4)	1363·6 (540·5–3979·4)
	G–CSF	408·1 ↓ *2 (157·9–709·6)	421·9 (157·9–709·6)	436·9 (62·2–1953·6)	300·0 (62·2–651·7)	550·0 ↑ *3 (213·7–2023·5)	497·6 (213·7–638·2)	425·1 (118·8–808·3)	424·0 (118·8–808·3)

Significant difference: *$P < 0·05$).

[1]In comparison with T1D group; [2]in comparison with CD group; [3]in comparison with control group.

IFN = interferon; IL = interleukin; MCP = monocyte chemoattractant protein; MIP = macrophage inflammatory protein; G–CSF = granulocyte–macrophage colony-stimulating factor.

Table 4. The serum level of acute-phase proteins, adipocytokines and matrix metalloproteinases (MMPs) in children with combined type 1 diabetes and celiac disease (T1D + CD), T1D, CD and reference children (control). Results are presented as median (minimum, maximum)

		T1D + CD		T1D		CD		Control	
	Immune markers (pg/ml)	Whole study cohort (18)	Cluster A (17)	Whole study cohort (27)	Cluster A (16)	Whole study cohort (16)	Cluster A (10)	Whole study cohort (42)	Cluster A (35)
Acute-phase proteins	Proalcitonin (PCT) [§]	248·0 (0·0–1808·8)	248·0 ↓ [*1] (0·0–1808·8)	892·2 ↑ [*2] (0·0–2397·7)	1170·2 ↑ [*2, *3] (0·0–2397·7)	0·0 (0·0–4502·6)	0·0 ↓ [*3] (0·0–592·0)	248·0 (0·0–2166·7)	428·3 (0·0–1808·8)
	Ferritin [§]	5233·2 (1818·5–47 444·2)	4999·0 (1818·5–41 367·9)	6169·3 (1771·1–34 616·7)	5262·4 (1937·3–11 475·2)	4676·7 (2352·0–34 616·7)	4530·1 (2352·0–18 865·9)	6198·5 (1699·6–53 520·8)	5291·8 (1699·6–53 520·8)
	Tissue plasminogen activator (tPA)[§]	800·1 (247·1–3276·7)	752·8 (247·1–3276·7)	1194·4 (0·0–2875·3)	957·9 (310·4–2502·3)	1283·2 (373·7–3823·3)	784·2 (373·7–2140·3)	1154·5 (156·2–5703·0)	1068·2 (310·4–5703·0)
	Fibrinogen	2685·0 ↓ [*1] (1612·4–8580·3)	2685·0 ↓ [*1] (1612·4–8580·3)	3356·0 (0·0–9866·0)	3268·2 (1937·7–5203·1)	2461·2 (1612·4–531 204·3)	2338·1 ↓ [*1, *3] (373·7–2140·3)	3566·2 (1197·6–13 219·9)	3268·2 (1197·6–13 219·9)
	Serum amyloid A (SAA)	207·6 (0·0–2145·3)	126·9 (0·0–2145·3)	342·0 (0·0–20 522·0)	310·0 (0·0–2335·2)	344·2 (0·0–5739·5)	0·0 ↓ [*1] (0·0–597·4)	155·9 (0·0–7498·2)	174·0 (0·0–7498·2)
Adipocytokines	Visfatin	446·5 ↓ [*1] (48·8–4890·2)	427·7 (48·8–3537·2)	2192·1 ↑ [*3] (0·0–257 408·9)	1950·3 (0·0–15 196·9)	455·7 (0·0–526 754·9)	309·5 (0·0–4890·0)	1016·0 (0·0–45 854·0)	897·2 (0·0–22 260·8)
	Resistin	4493·6 (2507·2–12 107·6)	4316·0 (2507·2–12 107·6)	6293·3 (2614·3–24 598·1)	5059·6 (2614·3–9478·2)	5465·9 (3943·1–13 240·0)	5038·4 (3943·1–7590·6)	5847·8 (2727·4–20 203·8)	5622·4 (2727·4–15 358·6)
MMPs	MMP-1	167·3 (24·0–1288·3)	167·3 (24·0–1288·3)	253·6 [*2] (29·2–1927·4)	262·4 (29·2–1927·4)	130·4 (45·1–418·7)	96·9 (45·1–418·7)	201·6 (3·9–1286·3)	214·3 (24·0–1286·3)
	MMP-2	41 886·1 (24 455·8–104 817·1)	42 197·5 ↓ [*3] (24 455·8–104 817·1)	38 522·1 ↓ [*3] (20 664·9–98 488·5)	41 285·7 ↓ [*3] (22 280·6–77 541·0)	35 577·5 (19 857·7–293 451·1)	35 577·5 (21 671·1–96 104·3)	51 148·6 (854·7–114 846·3)	52 043·9 (20 917·0–114 846·3)
	MMP-3	888·1 (48·3–6662·6)	920·0 (48·3–6662·6)	1267·8 ↑ [*2] (275·0–20 421·1)	1202·0 ↑ [*2] (342·8–20 421·1)	740·4 (117·0–5722·5)	484·6 ↓ [*3] (484·6–1400·6)	1067·8 (153·0–5742·6)	1089·0 (382·1–5742·6)

*Significant difference ($P < 0·05$); [§]ng/ml.

[1]In comparison with T1D group; [2]in comparison with CD group; [3]in comparison with control group.

MMP = Matrix metallloproteinase.

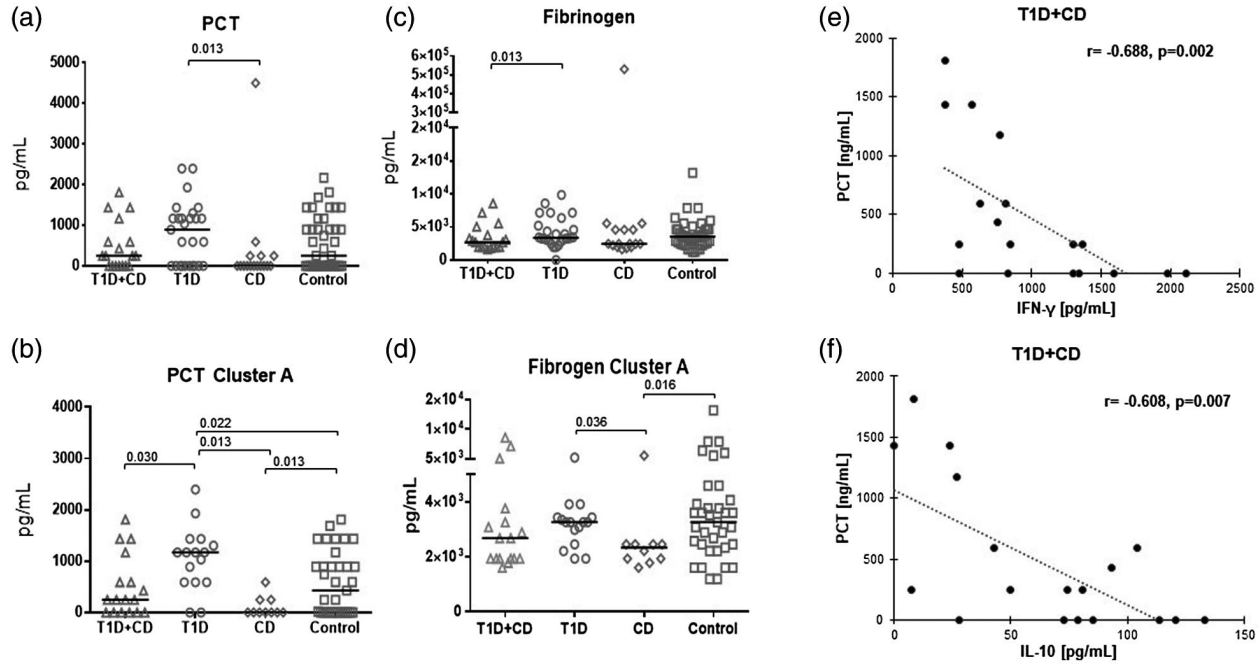

Fig. 3. Serum levels of acute-phase proteins in children with combined type 1 diabetes and celiac disease (T1D + CD, $n = 18$), T1D ($n = 27$), CD ($n = 16$) and reference children (control, $n = 42$). (a) Procalcitonin (PCT), (c) fibrinogen in the whole study cohort and (b) PCT, (d) fibrinogen within cluster A. Correlation between PCT, (e) interferon (IFN)-γ and (f) interleukin (IL)-10 in children with combined T1D and CD in the whole study cohort.

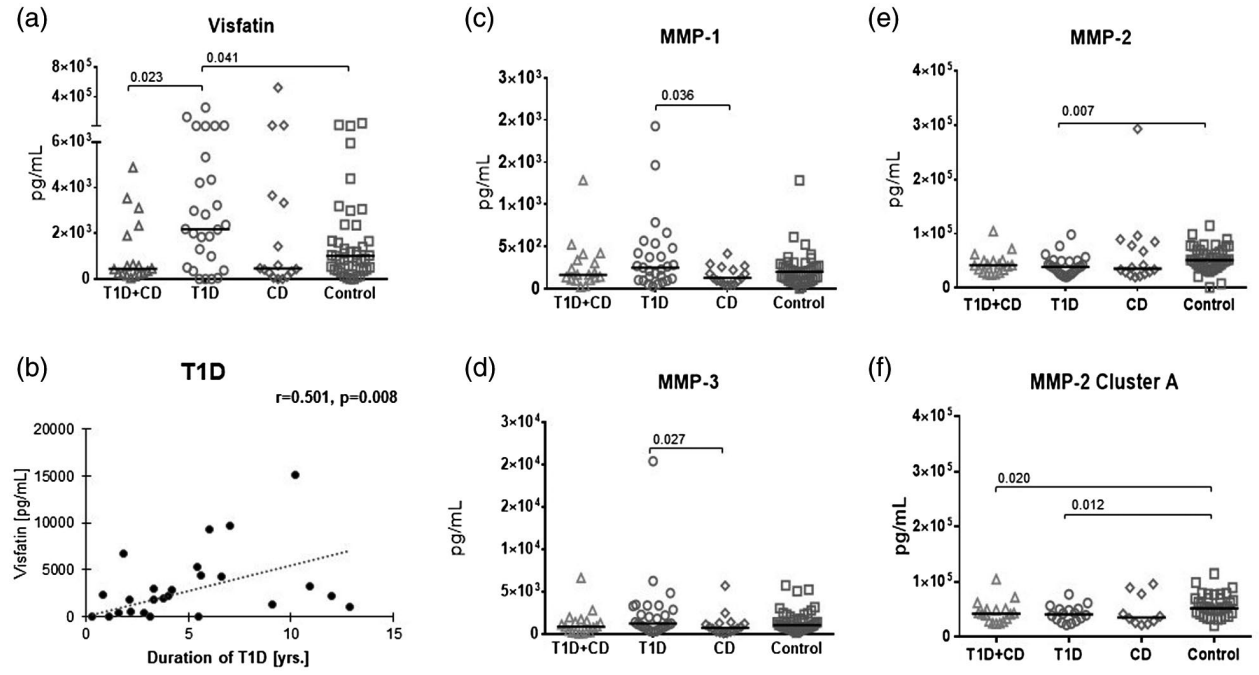

Fig. 4. Serum levels of (a) the adipocytokine visfatin, (c) matrix metalloproteinase (MMP)-1, (e) MMP-2, (d) MMP-3 in children with combined type 1 diabetes and celiac disease (T1D + CD, $n = 18$), T1D ($n = 27$), CD ($n = 16$) and reference children (control, $n = 42$) in the whole study cohort; and (f) MMP-2 within cluster A. Correlation between (b) visfatin levels and duration of T1D in children with exclusively T1D.

CD alone or in combination with T1D compared to children with T1D, observed both in the whole study cohort ($P < 0{\cdot}05$, Fig. 3a) as well as in cluster A ($P < 0{\cdot}05$, Fig. 3b). In children with a double diagnosis, contrary to the other groups, the serum levels of PCT were inversely correlated to IFN-γ ($r = -0{\cdot}688$, $P < 0{\cdot}01$, Fig. 4e) and IL-10

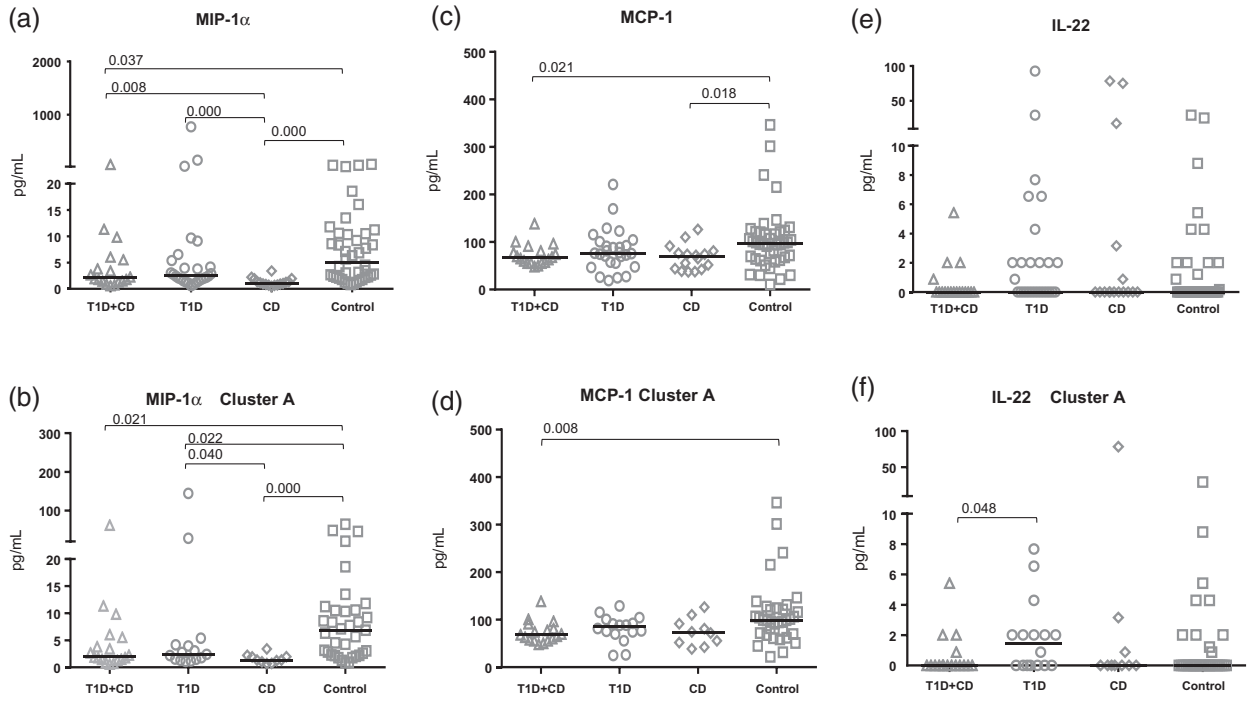

Fig. 5. Serum levels of cytokines and chemokines in children with combined type 1 diabetes and celiac disease (T1D + CD, $n = 18$), T1D($n = 27$), CD ($n = 16$) and reference children (control, $n = 42$). (a) Macrophage inflammatory proteins (MIP)-1α, (c) monocyte chemoattractant protein (MCP)-1, (e) interleukin (IL)-22 in the whole study cohort; and (b) MIP-1α, (d) MCP-1 and (f) IL-22 in cluster A.

($r = -0{\cdot}608$, $P < 0{\cdot}01$, Fig. 3f) (equally distributed within the whole study cohort and cluster A). Children diagnosed with combined T1D and CD also had lower levels of fibrinogen ($P < 0{\cdot}05$, Fig. 3c) and visfatin ($P < 0{\cdot}05$, Fig. 4a) compared with children diagnosed exclusively with T1D. Further, children with a double diagnosis had lower serum levels of MMP-2 in comparison to the control group ($P < 0{\cdot}05$, Fig. 4d) as well as lower levels of IL-22 compared to children with T1D ($P < 0{\cdot}05$). In summary, children diagnosed with T1D in combination with CD had a diminished secretion of several cytokines, chemokines, adipocytokines, APPs and MMPs (summarized in Fig. 2).

Discussion

A role for soluble immune markers in the pathogenesis of many autoimmune diseases is well established. The immunological imbalance of the immune system seen, for example, in patients with both T1D and CD is dominated by a dysregulated Th1 response together with changes in secretion of different immune markers. Several studies report inconsistent results on up- and/or down-regulation of several immune markers in T1D and CD, respectively [12,14,17,50,51]. However, the peripheral immunoregulatory milieu is not well studied in children diagnosed with a combination of these diseases.

In this study, we have examined 28 peripheral immune markers in children diagnosed with T1D and/or CD or without these autoimmune diseases. First, differences between the diagnosis groups were examined in the whole study cohort, indicating differences in the concentrations of IL-10, IL-22, MIP-1α, MIP-1β, IL-8, MCP-1, G-CSF, PCT, visfatin, MMPs and fibrinogen. To further identify possible similarities/differences in immune marker profiles and to gain a deeper understanding of the peripheral immunological milieu in the different patient groups, HCA was performed. HCA has been shown to be adequate to identify even small, but important, changes in immune marker levels [49]. Contrary to conventional analysis, this approach did not divide the samples into a priori groups, but aggregated the results based on similarity to each other. Regarding the clustering pattern, the results also elucidated a possible association between the concentration of immune markers and the age of participants. The divergences seen in the age pattern between the clusters in T1D, CD and references probably reflect the previously described age-associated changes in immune marker levels [52]. The cluster analysis, in comparison to analysis of the

whole study cohort, confirmed differences in concentrations of the immune markers between the groups of children with T1D and/or CD.

Th1-associated IFN-γ is a crucial mediator in developing cell destruction in both T1D and CD [53–55]. In line with results from previous studies [14,17], our results showed higher (non-significant) levels of IFN-γ in children with T1D or CD in comparison to reference children. This discrepancy could probably partly depend upon biological variation, e.g. disease activity, duration of disease and age of participants, and partly on the variation in the number of participants between the different studies [12,14,17,50,51].

Regarding anti-tTG IgA status, 100% within the T1D group, 81% within the CD group (13 of 16) and 67% in the double diagnosis group (12 of 18) were anti-tTG-IgA-negative. Recent studies have shown that tTG serology is poorly correlated with mucosal outcomes and should not be considered as an indicator of mucosal healing [56–58]. As all children were on a gluten-free diet for at least 3 months after diagnosis before inclusion in this study we have included all children diagnosed with CD, regardless of anti-tTG IgA status (three children with CD and six with double diagnosis were anti-tTG IgA-positive). To clarify if these anti-tTG IgA-positive children had influenced our results, we examined differences between the different study groups (i.e. the whole cohort as well as in the clustered groups) in relation to tTG-status. However, we did not observe that children with positive tTG-IgA influenced the results. Thus, we consider that children with positive anti-tTG IgA status and supposedly poor gluten-free diet compliance were acceptable for inclusion in the study population.

With the exception of IL-22, we did not find significant differences among the Th17 cytokines (IL-17A, IL-25, IL-33) between the different study groups. IL-22 is characterized by a dual nature and, depending on environmental factors, has protective or inflammatory activity during the immune response [59]. Children with T1D within cluster A showed higher levels of IL-22 in comparison to children with a double diagnosis. We assume that this can depend upon the discrepancies in diabetes duration (Table 2) as increased Th22 responses have been seen, especially during the early stages of T1D (< 5 years) [6]. Interestingly, the levels of IL-22 in children with a double diagnosis is in line with the levels observed in children with CD as well as in the reference group. These data may suggest a possible protective effect of IL-22, which can probably contribute to preventing the development of CD in people with T1D.

Results concerning MCP-1, MIP-1α and MIP-1β in T1D and/or CD are limited and conflicting [17,60–62]. We observed lower levels of IL-8, MCP-1, MIP-1α and MIP-1β in children diagnosed with CD with or without T1D compared to reference children. Vorobjova *et al*. showed alterations in cytokine and chemokine levels and correlation of cytokine/chemokine levels with the degree of small bowel mucosa damage in children with active disease and also to age [60]. We suggest that the divergence in results could depend upon differences in the inflammation stage in the mucosa and also on the age of participants.

The relationship between autoimmune diseases, low-grade inflammation and APPs is still unclear. However, low-grade chronic inflammation and stimulation of the innate immune system were identified as contributing to the pathogenesis of both T1D and CD [22,23]. In this study, we examined the serum levels of PTC, ferritin, tPA, fibrinogen and SAA. Procalcitonin is known as a systemic inflammatory protein, induced by various inflammatory markers such as IL-6 and TNF-α, but the physiological role still remains uncertain. Previous studies present minor elevated PCT levels in autoimmune diseases [63]. To our knowledge, no studies have evaluated the PCT levels in the context of T1D and/or CD. Interestingly, our results showed lower levels of PCT in children with a combined diagnosis in comparison with the group of children with T1D exclusively. Another aspect of interest concerns the lower levels of PCT in children with CD compared to reference children and children with T1D, respectively. Our reflection is that PCT could (possibly) have a role in immunological processes associated with CD that contribute to the reduced PCT levels seen in CD as well as in the context of double diagnosis. Similarly to previous studies, we found that children with T1D, and also in combination with CD, had lower serum levels of IL-10 in comparison to CD children [13,38] and children with CD have higher IL-10 levels compared to reference children [14,17,60]. In children diagnosed with both T1D and CD, in contrast to the other studied groups, PCT were inversely correlated to secretion of IL-10. We suppose that the reduced levels of IL-10 observed in children with a double diagnosis is primarily associated with the dysregulated suppressive process seen in T1D. Based on these findings, PCT together with IL-10 may be of interest as a prognostic marker for development of CD in children with T1D.

In recent years, more focus has been directed to the anti-apoptotic activity and regulatory role in inflammation of adipocytokines, notably visfatin. Studies have identified changes in the circulatory levels of visfatin in autoimmune diseases and highlighted it as a molecule of clinical relevance that could be an interesting biomarker with possible prognostic significance, and possibly as a potential therapeutic target [36,64–66]. Previous studies have shown an existing relationship between T1D and visfatin, but the mechanism is not fully understood. However, to our knowledge, no studies have evaluated the serum levels of visfatin either in children with both

diagnoses (T1D and CD) or in children with CD exclusively. Few studies have evaluated circulating visfatin in T1D, and those reported either decreased [67–69] or increased levels [70]. Interestingly, in the present study, we found higher levels of visfatin in T1D children compared to both children with a double diagnosis and reference children. We suggest possible explanations for these results, which indicate differences in the levels of visfatin between children with T1D exclusively and children diagnosed with both T1D and CD. First, visfatin could be involved in the pathogenesis of CD supported by Waluga *et al*. that showed a relationship between visfatin and intestinal inflammatory disease, which share genetics and functional pathways with CD [71]. Secondly, it has previously been demonstrated that diminished regulatory activity in immune cells is related to increased serum adipocytokine levels in inflammatory disease [64]. In a previous study, we observed diminished regulatory T cell activity in T_{reg} cells in children with T1D but not in children with both T1D and CD. Based on these findings, we suggest that visfatin may possibly play a significant role, eventually contributing to protection from development of CD in children with already-diagnosed T1D.

Several studies suggest that MMPs are important contributors in the degradation of extracellular matrix in disorders characterized by intestinal tissue destruction, e.g. CD [39,41]. An association between increased expression of MMP-1 and MMP-3 and the stage of inflammation in the gut in patients with T1D has been reported [41, 72]. This could partly explain our findings of higher levels of MMP-1 and MMP-3 in children diagnosed with T1D.

Taken together, our results contribute to an increased understanding of the peripheral immunoregulatory milieu in T1D and CD. We conclude that besides cytokines and chemokines, other immune markers, e.g. APPs, MMPs and adipocytokines, are also of importance for further investigations to elucidate the heterogeneous immune processes in a combined diagnosis of T1D and CD. As the possible pathophysiological roles of several studied immune markers in the context of a double diagnosis of T1D and CD are unclear, functional studies to elucidate the interrelationships between these immune markers and the pathophysiology in the double diagnosis of these autoimmune diseases are necessary.

Disclosures

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Author contributions

The author contributions are as follows: M. F. was the principal investigator of this paper and designed the study together with K. Å. K. Å. was the medical adviser and responsible for contact with all patients and healthy children included in the study. A. T., a PhD student, was responsible for laboratory analysis and data analysis. S. K. contributed to HCA analyses. All authors contributed to the interpretation of the results and preparation of this manuscript.

References

1 Jabri B, Kasarda DD, Green PHR. Innate and adaptive immunity: the Yin and Yang of celiac disease. Immunol Rev 2005; **206**:219–31.
2 Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nat Rev Immunol 2013; **13**:294–302.
3 Knip M, Siljander H. Autoimmune mechanisms in type 1 diabetes. Autoimmun Rev 2008; **7**:550–7.
4 Nilsson L, Kivling A, Jalmelid M, Falth Magnusson K, Faresjo M. Combinations of common chronic paediatric diseases deviate the immune response in diverging directions. Clin Exp Immunol 2006; **146**:433–42.
5 Honkanen J, Nieminen JK, Gao R *et al*. IL-17 immunity in human type 1 diabetes. J Immunol 2010; **185**:1959–67.
6 Xu X, Zheng S, Yang F *et al*. Increased Th22 cells are independently associated with Th17 cells in type 1 diabetes. Endocrine 2014; **46**:90–8.
7 Walker LS, von Herrath M. CD4 T cell differentiation in type 1 diabetes. Clin Exp Immunol 2016; **183**:16–29.
8 Ryba-Stanislawowska M, Werner P, Brandt A, Mysliwiec M, Mysliwska J. Th9 and Th22 immune response in young patients with type 1 diabetes. Immunol Res 2016; **64**:730–5.
9 Hussain MJ, Peakman M, Gallati H *et al*. Elevated serum levels of macrophage-derived cytokines precede and accompany the onset of IDDM. Diabetologia 1996; **39**:60–9.
10 Kallmann BA, Hüther M, Tubes M *et al*. Systemic bias of cytokine production toward cell-mediated immune regulation in IDDM and toward humoral immunity in Graves disease. Diabetes 1997; **46**:237–43.
11 Alnek K, Kisand K, Heilman K, Peet A, Varik K, Uibo R. Increased blood levels of growth factors, proinflammatory cytokines, and Th17 cytokines in patients with newly diagnosed type 1 diabetes. PLOS ONE 2015;**10**;e0142976.
12 Chatzigeorgiou A, Harokopos V, Mylona-Karagianni C, Tsouvalas E, Aidinis V, Kamper E. The pattern of inflammatory/anti-inflammatory cytokines and chemokines in type 1 diabetic patients over time. Ann Med 2010; **42**:426–38.

13 Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TI. Defective suppressor function in CD4(+)CD25(+) T-cells from patients with type 1 diabetes. Diabetes 2005; **54**:92–9.

14 Manavalan JS, Hernandez L, Shah JG *et al*. Serum cytokine elevations in celiac disease: association with disease presentation. Hum Immunol 2010; **71**:50–7.

15 Nilsen EM, Jahnsen FL, Lundin KE *et al*. Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. Gastroenterology 1998; **115**:551–63.

16 Hansson T, Dannaeus A, Klareskog L. Cytokine-producing cells in peripheral blood of children with coeliac disease secrete cytokines with a type 1 profile. Clin Exp Immunol 1999; **116**:246–50.

17 Bjorck S, Lindehammer SR, Fex M, Agardh D. Serum cytokine pattern in young children with screening detected coeliac disease. Clin Exp Immunol 2015; **179**:230–5.

18 Pender SL, Tickle SP, Docherty AJ, Howie D, Wathen NC, MacDonald TT. A major role for matrix metalloproteinases in T cell injury in the gut. J Immunol 1997; **158**:1582.

19 Mauviel A. Cytokine regulation of metalloproteinase gene expression. J Cell Biochem 1993; **53**:288–95.

20 Castellanos-Rubio A, Santin I, Irastorza I, Castano L, Carlos Vitoria J, Ramon Bilbao J. TH17 (and TH1) signatures of intestinal biopsies of CD patients in response to gliadin. Autoimmunity 2009; **42**:69–73.

21 Lahdenpera AI, Falth-Magnusson K, Hogberg L, Ludvigsson J, Vaarala O. Expression pattern of T-helper 17 cell signaling pathway and mucosal inflammation in celiac disease. Scand J Gastroenterol 2014; **49**:145–56.

22 Gomes MB, Piccirillo LJ, Nogueira VG, Matos HJ. Acute-phase proteins among patients with type 1 diabetes. Diabetes Metabolism 2003; **29**(4 Pt 1):405–11.

23 Tetzlaff WF, Meroño T, Menafra M *et al*. Markers of inflammation and cardiovascular disease in recently diagnosed celiac disease patients. World J Cardiol 2017; **9**:448–56.

24 Roep BO. The role of T-cells in the pathogenesis of Type 1 diabetes: from cause to cure. Diabetologia 2003; **46**:305–21.

25 Przemioslo RT, Kontakou M, Nobili V, Ciclitira PJ. Raised pro-inflammatory cytokines interleukin 6 and tumour necrosis factor alpha in coeliac disease mucosa detected by immunohistochemistry. Gut 1994; **35**:1398–403.

26 Wiedermann FJ, Kaneider N, Egger P *et al*. Migration of human monocytes in response to procalcitonin. Crit Care Med 2002; **30**:1112–7.

27 Torti FM, Torti SV. Regulation of ferritin genes and protein. Blood 2002; **99**:3505–16.

28 Kell DB, Pretorius E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics 2014; **6**:748–73.

29 Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. J Immunol 2001; **167**:2887.

30 Hodgkinson CP, Patel K, Ye S. Functional Toll-like receptor 4 mutations modulate the response to fibrinogen. Thromb Haemost 2008; **100**:301–7.

31 Hatanaka E, Furlaneto C, Ribeiro F, Souza G, Campa A. Serum amyloid A-induced MRNA expression and release of tumor necrosis factor-alpha (TNF-α) in human neutrophils. Immunol Lett 2004; **91**:33–7.

32 Lin L, Hu K. Tissue plasminogen activator and inflammation: from phenotype to signaling mechanisms. Am J Clin Exp Immunol 2014; **3**:30–6.

33 Polepalle T, Moogala S, Boggarapu S, Pesala DS, Palagi FB. Acute phase proteins and their role in periodontitis. A review. J Clin Diagn Res 2015; **9**:ZE01-ZE5.

34 Moschen AR, Kaser A, Enrich B *et al*. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 2007; **178**:1748–58.

35 Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. J Immunol 2005; **174**:5789–95.

36 Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol 2006; **6**:772–83.

37 Xiao K, Zou WH, Yang Z *et al*. The role of visfatin on the regulation of inflammation and apoptosis in the spleen of LPS-treated rats. Cell Tissue Res 2015; **359**:605–18.

38 Filkova M, Haluzik M, Gay S, Senolt L. The role of resistin as a regulator of inflammation: implications for various human pathologies. Clin Immunol 2009; **133**:157–70.

39 Nissinen L, Kahari VM. Matrix metalloproteinases in inflammation. Biochem Biophys Acta 2014; **1840**:2571–80.

40 Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 2008; **19**:34–41.

41 Pender SL, Tickle SP, Docherty AJ, Howie D, Wathen NC, MacDonald TT. A major role for matrix metalloproteinases in T cell injury in the gut. J Immunol 1997; **158**:1582–90.

42 Parks WC, Wilson CL, López-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004; **4**:617–29.

43 Thrailkill KM, Bunn RC, Moreau CS *et al*. Matrix metalloproteinase-2 dysregulation in type 1 diabetes. Diabetes Care 2007; **30**:2321–6.

44 Gharagozlian S, Svennevig K, Bangstad H-J, Winberg J-O, Kolset SO. Matrix metalloproteinases in subjects with type 1 diabetes. BMC Clin Pathol 2009; **9**:7.

45 Daum S, Bauer U, Foss HD *et al*. Increased expression of mRNA for matrix metalloproteinases-1 and -3 and tissue inhibitor of metalloproteinases-1 in intestinal biopsy specimens from patients with coeliac disease. Gut 1999; **44**:17–25.

46 Craig ME, Hattersley A, Donaghue KC. Definition, epidemiology and classification of diabetes in children and adolescents. Ped Diabetes 2009; **10**(Suppl 12):3–12.

47 Walker-Smith J, Guandalini S, Schmitz J, Shmerling D, Visakorpi J. Revised criteria for diagnosis of coeliac disease. Report of working group of European Society of Paediatric Gastroenterology and Nutrition. Arch Dis Child 1990; **65**:909–11.

48 Tighe PJ, Ryder RR, Todd I, Fairclough LC. ELISA in the multiplex era: Potentials and pitfalls. Proteom Clin Appl 2015; **9**:406–22.
49 Yang C, Du H. Evaluation by hierarchical clustering of multiple cytokine expression after phytohemagglutinin stimulation. Archiv Biol Sci 2016; **68**:40.
50 Lahat N, Shapiro S, Karban A, Gerstein R, Kinarty A, Lerner A. Cytokine profile in coeliac disease. Scand J Immunol 1999; **49**:441–6.
51 Kaas A, Pfleger C, Kharagjitsingh AV *et al*. Association between age, IL-10, IFNγ, stimulated C-peptide and disease progression in children with newly diagnosed Type 1 diabetes. Diabet Med 2012; **29**:734–41.
52 Decker ML, Gotta V, Wellmann S, Ritz N. Cytokine profiling in healthy children shows association of age with cytokine concentrations. Sci Rep 2017; **7**:17842.
53 Shan L, Molberg O, Parrot I *et al*. Structural basis for gluten intolerance in celiac sprue. Science 2002; **297**:2275–9.
54 Yi Z, Li L, Garland A *et al*. IFN-γ receptor deficiency prevents diabetes induction by diabetogenic CD4+, but not CD8+, T cells. Euro J Immunol 2012; **42**:2010–8.
55 Borrelli M, Salvati VM, Maglio M *et al*. Immunoregulatory pathways are active in the small intestinal mucosa of patients with potential celiac disease. Am J Gastroenterol 2013; **108**:1775–84.
56 Comino I, Fernandez-Banares F, Esteve M *et al*. Fecal gluten peptides reveal limitations of serological tests and food questionnaires for monitoring gluten-free diet in celiac disease patients. Am J Gastroenterol 2016; **111**:1456–65.
57 Webb C, Myleus A, Norstrom F *et al*. High adherence to a gluten-free diet in adolescents with screening-detected celiac disease. J Pediatr Gastroenterol Nutr 2015; **60**:54–9.
58 Silvester JA, Kurada S, Szwajcer A, Kelly CP, Leffler DA, Duerksen DR. Tests for serum transglutaminase and endomysial antibodies do not detect most patients with celiac disease and persistent villous atrophy on gluten-free diets: a meta-analysis. Gastroenterology 2017; **153**:689–701.e1.
59 Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. Immunity 2008; **29**:947–57.
60 Vorobjova T, Tagoma A, Oras A *et al*. Celiac disease in children, particularly with accompanying type 1 diabetes, is characterized by substantial changes in the blood cytokine balance, which may reflect inflammatory processes in the small intestinal mucosa. J Immunol Res 2019; **2019**:17.
61 Guan R, Purohit S, Wang H *et al*. Chemokine (C-C motif) ligand 2 (CCL2) in sera of patients with type 1 diabetes and diabetic complications. PLOS ONE 2011; **6**;e17822.
62 Purohit S, Sharma A, Hopkins D *et al*. Large-scale discovery and validation studies demonstrate significant reductions in circulating levels of IL8, IL-1Ra, MCP-1, and MIP-1β in patients with type 1 diabetes. J Clin Endocrinol Metab 2015; **100**:E1179–87.
63 Meisner M. Update on procalcitonin measurements. Ann Lab Med 2014; **34**:263–73.
64 Emamgholipour S, Eshaghi SM, Hossein-nezhad A, Mirzaei K, Maghbooli Z, Sahraian MA. Adipocytokine profile, cytokine levels and foxp3 expression in multiple sclerosis: a possible link to susceptibility and clinical course of disease. PLOS ONE 2013; **8**;e76555-e.
65 Romacho T, Villalobos LA, Cercas E, Carraro R, Sanchez-Ferrer CF, Peiro C. Visfatin as a novel mediator released by inflamed human endothelial cells. PLOS ONE 2013; **8**:e78283.
66 Lu GW, Wang QJ, Xia MM, Qian J. Elevated plasma visfatin levels correlate with poor prognosis of gastric cancer patients. Peptides 2014; **58**:60–4.
67 Toruner F, Altinova AE, Bukan N *et al*. Plasma visfatin concentrations in subjects with type 1 diabetes mellitus. Horm Res 2009; **72**:33–7.
68 Alexiadou K, Kokkinos A, Liatis S, Perrea D, Katsilambros N, Tentolouris N. Differences in plasma apelin and visfatin levels between patients with type 1 diabetes mellitus and healthy subjects and response after acute hyperglycemia and insulin administration. Hormones 2012; **11**:444–50.
69 El Samahi MH, Ismail NA, Matter RM, Selim A, Ibrahim AA, Nabih W. Study of visfatin level in type 1 diabetic children and adolescents. Open Access Maced J Med Sci 2017; **5**:299–304.
70 Haider DG, Pleiner J, Francesconi M, Wiesinger GF, Muller M, Wolzt M. Exercise training lowers plasma visfatin concentrations in patients with type 1 diabetes. J Clin Endocrinol Metab 2006; **91**:4702–4.
71 Waluga M, Hartleb M, Boryczka G, Kukla M, Zwirska-Korczala K. Serum adipokines in inflammatory bowel disease. World J Gastroenterol 2014; **20**:6912–7.
72 Bister V, Kolho K-L, Karikoski R, Westerholm-Ormio M, Savilahti E, Saarialho-Kere U Metalloelastase (MMP-12) is upregulated in the gut of pediatric patients with potential celiac disease and in type 1 diabetes. Scand J Gastroenterol 2005;**40**:1413–1422. https://pubmed.ncbi.nlm.nih.gov/16293556/.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Cut-off values for the analyzed immune markers.

