Distinct Monocyte Gene-Expression Profiles in Autoimmune Diabetes

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OBJECTIVE—There is evidence that monocytes of patients with type 1 diabetes show proinflammatory activation and disturbed migration/adhesion, but the evidence is inconsistent. Our hypothesis is that monocytes are distinctly activated/disturbed in different subforms of autoimmune diabetes.

RESEARCH DESIGN AND METHODS—We studied patterns of inflammatory gene expression in monocytes of patients with type 1 diabetes (juvenile onset, $n = 30$; adult onset, $n = 30$) and latent autoimmune diabetes of the adult (LADA) ($n = 30$) (controls subjects, $n = 49$; type 2 diabetic patients, $n = 30$) using quantitative PCR. We tested 25 selected genes: 12 genes detected in a prestudy via whole-genome analyses plus an additional 13 genes identified as part of a monocyte inflammatory signature previously reported.

RESULTS—We identified two distinct monocyte gene expression clusters in autoimmune diabetes. One cluster (comprising 12 proinflammatory cytokine/compound genes with a putative key gene *PDE4B*) was detected in 60% of LADA and 28% of adult-onset type 1 diabetic patients but in only 10% of juvenile-onset type 1 diabetic patients. A second cluster (comprising 10 chemotaxis, adhesion, motility, and metabolism genes) was detected in 43% of juvenile-onset type 1 diabetic and 33% of LADA patients but in only 9% of adult-onset type 1 diabetic patients.

CONCLUSIONS—Subgroups of type 1 diabetic patients show an abnormal monocyte gene expression with two profiles, supporting a concept of heterogeneity in the pathogenesis of autoimmune diabetes only partly overlapping with the presently known diagnostic categories. ***Diabetes*** **57:2768–2773, 2008**

There is evidence that monocytes of patients with type 1 diabetes are functionally aberrant, showing raised production of interleukin (IL)-1β, IL-6, superoxide anion, and prostaglandin-endoperoxide synthase 2 (PTGS2) (1–3); aberrant generation of antigen-presenting cells (4,5); and abnormal chemotaxis, adhesion, and migratory potential (6). These aberrancies are thought to play a role in the pathogenesis of the disease by disrupting tolerance and aggravating the β-cell cytotoxic potential of infiltrating monocyte-derived dendritic cells and macrophages. However, these aberrant functional findings could not always be reproduced, particularly with regard to the enhanced production of PTGS2 (7) and the poor generation of antigen-presenting cells from monocytes (8). Two issues could be relevant to these discrepancies. First, raised production of proinflammatory monocyte-derived cytokines could be related to hyperglycemia (9). Second, there might be heterogeneity within autoimmune diabetes, such as has been noted previously between adult and juvenile forms of type 1 diabetes on the basis of genetic, immune, and metabolic characteristics (10). This possible heterogeneity in autoimmune diabetes might also become evident in different monocyte activation profiles.

To resolve these issues, we focus here on patterns of inflammatory gene expression in monocytes from selected patients distinguished by clinical characteristics and age at diagnosis, as well as from control subjects. Our hypothesis is that monocytes might be distinctly activated and disturbed within the known diagnostic categories of diabetes.

Recently, we reported a signature of 18 inflammatory-related genes in monocytes of bipolar patients (11); activated monocytes are thought to play a role in the pathogenesis of bipolar disorder (12,13). Given the reported association between bipolar disorder and autoimmune diabetes (14), and given the possible central role of monocytes in both disorders, we tested this set of 18 proinflammatory monocyte genes in patients with autoimmune diabetes. To these 18 monocyte genes, we added 7 genes identified in a whole-genome expression profile of a set of juvenile-onset type 1 diabetic patients who had been compared with healthy control subjects and type 2 diabetic patients (see supplementary Fig. 1 [available in an online appendix at http://dx.doi.org/10.2337/db08-0496]). Thus, using quantitative RT-PCR (Q-PCR), we validated abnormal expression of 25 monocyte activation genes in latent autoimmune diabetes of the adult (LADA), adult-onset type 1 diabetic and juvenile-onset type 1 diabetic patients, and, as controls, type 2 diabetic patients and healthy subjects.

RESEARCH DESIGN AND METHODS

All participants were diagnosed with diabetes according to the criteria of the American Diabetes Association (15). The characteristics of both patients and control subjects are shown in a supplementary Table A.

The methods of blood collection and storage, preparation of purified $CD14^+$ monocytes, mRNA isolation, and Q-PCR have been described in detail elsewhere (11) and are given in the legend of supplementary Table

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B. Statistics used have been given in the legends of the various tables and figures.

RESULTS

Q-PCR analysis. Table 1 shows the gene expression levels of 24 of 25 selected genes in the monocytes of the tested diabetic groups. These 25 genes comprised 12 genes (10 upregulated and 2 downregulated), identified in a prestudy (Affymetrix gene expression profiling, supplementary Fig. 1), that differentiated type 1 diabetes monocytes from both type 2 diabetes and control monocytes (fourfold difference, $P < 0.01$). These 12 genes were *STX1A*, *DHRS3 (SDR1)*, *FABP5*, *CD9*, *CDC42*, *chemokine ligand 2 (CCL2)*, *CCL7* (*MCP-3*), *PTPN7*, *NAB2*, and *EMP1* (all upregulated) and *BAZ1A* and *HSPA1A* (each downregulated). The other 13 genes we tested (*PDE4B*, *IL1B*, *IL6*, *tumor necrosis factor (TNF)*, *PTGS2*, *pentraxin 3 (PTX3)*, *CCL20*, *CXCL2*, *MAPK6*, *DUSP2*, *ATF3*, *TNFAIP3*, and *BCL2A1*) were reported elsewhere (11) as a coherent and mutually correlating set (signature) of 18 aberrantly expressed inflammation-related genes in monocytes of bipolar patients; 5 of the 18 genes, i.e., *CDC42*, *CCL2*, *CCL7*, *NAB2*, and *EMP1*, were also detected in our Affymetrix prestudy in purified type 1 diabetes monocytes.

In Table 1, data are given as relative fold changes, a method that is widely used but that has potential limitations (e.g., less accurate for genes with a large difference from the reference gene; for raw cycle threshold values see supplementary Table C), as does our standardization of patient data to control subjects (which was done to correct for the observed interassay variation). To address the latter issue, data were also analyzed before standardization to control subjects (supplementary Table D). In essence, the same conclusions can be drawn from both analyses: *1*) 24 of 25 studied genes were validated as aberrantly expressed (*BAZ1A* was not abnormally expressed); *2*) although monocytes of juvenile-onset type 1 diabetic, adult-onset type 1 diabetic, LADA, and type 2 diabetic patients all showed enhanced gene expression of many of the inflammatory genes compared with control subjects, they also showed differences compared with each other; and *3*) some of the genes were specific for a diagnostic category. The upregulation of *PDE4B*, *TNFAIP3*, and *MAPK6* were specific for LADA monocytes; the upregulation of *FABP5* and the downregulation of *HSPA1A* were specific for juvenile-onset type 1 diabetes monocytes. Neither adult-onset type 1 diabetes nor type 2 diabetes had an up- or downregulation of a specific gene, although type 2 diabetes monocytes showed a clear upregulation of many of the inflammatory genes.

The gene expression levels within each subject group did not correlate with A1C, BMI, age, sex, age at onset of diabetes, or disease duration (tested by ANCOVA). To further analyze the data, we embarked on cluster analysis.

Identification of two gene expression clusters and their presence in LADA, type 1 diabetic, and type 2 diabetic patients. Figure 1 shows the Q-PCR data of the patients and control subjects in hierarchical cluster analysis. The dendrogram of average linkage showed two interdependent main gene clusters.

In another and different cluster analysis of the Q-PCR data, we correlated the expression levels of the 24 abnormally expressed genes to the expression level of the following: *1*) *PDE4B*, because it is one of the genes specific for LADA and a putative key gene for cluster 1 (see DISCUSSION), and *2*) *FABP5*, because this cluster 2 gene is specific for juvenile-onset type 1 diabetes. Table 2 shows that around these specific genes, two mutually correlating gene expression sets appeared. In the *PDE4B*-correlating set, all cluster 1 and cluster 2 genes (apart from *FABP5*) were present and correlated strongly with the gene expression of *PDE4B*. In the *FABP5*-correlating set, almost all cluster 2 genes were present (except for *NAB2*), along with *CXCL2*, *PTGS2*, *HSPA1A*, and *CD9*. Interestingly, *PTGS2* was overexpressed in the *PDE4B*-positive subjects, whereas its expression was reduced in *FABP5*-positive subjects (Table 2).

We next sought the relationship of different patient groups to cluster 1 and cluster 2 genes (Table 3). Cluster 1 and the *PDE4B*-correlating set were significantly more frequent in adult-onset type 1 diabetic, LADA, and type 2 diabetic patients compared with control subjects, whereas control subjects and juvenile-onset type 1 diabetic patients were similar in this regard. Cluster 2 and the *FABP5*-correlating set were significantly more frequent in both juvenile-onset type 1 diabetic and LADA patients compared with control subjects, adult-onset type 1 diabetic, and type 2 diabetic patients. Neither clusters nor specific *PDE4B* or *FABP5* gene expression were related to age, A1C, glucose level, or BMI within any of the groups studied.

Correlations between gene expression levels in circulating monocytes and serum levels of cytokines. In addition to monocyte gene analysis, we determined serum levels of IL-6, tumor necrosis factor–α, pentraxin 3 (PTX3), and CCL2 in patients and control subjects (for data see supplementary Fig. 2) and correlated gene expression levels to corresponding serum cytokine levels. Monocyte gene expression levels of *PTX3* and *IL6* (*PTX3*: $r = 0.26$, $P = 0.004$; *IL-6*: $r = 0.23$, $P = 0.034$; Spearman's correlation), but not of *TNF* and *CCL2*, correlated with serum protein levels. A possible explanation for this observed discrepancy between mRNA and protein expression levels is that serum levels of cytokines are more subject to confounders (e.g., BMI, glucose levels) than gene expression levels, as is suggested by our data (supplementary Fig. 2).

We also compared the serum cytokine levels of cluster-positive and cluster-negative patients. We found higher serum levels of PTX3 in cluster 1–positive compared with cluster 1–negative patients (Fig. 2), suggesting an in vivo relevance of at least cluster 1 gene expression. Elevated levels of serum PTX3, a novel acute phase protein, have been found in other autoimmune conditions such as rheumatoid arthritis and scleroderma (16).

DISCUSSION

This study shows two distinct monocyte gene-expression profiles in autoimmune diabetes, indicating different activation profiles, which suggests heterogeneity in the pathogenesis of autoimmune diabetes.

We identified one profile of mainly proinflammatory genes (*IL1B*, *IL6*, *TNF*, *PTGS2*, *PTX3*, *CCL20*, *CXCL2*, *DUSP2*, *ATF3*, *TNFAIP3*, and *BCL2A1*) with a putative key gene *PDE4B*. PDE4B is a c-AMP–degrading enzyme and could be a key molecule for turning monocytes into high proinflammatory cytokine–producing cells, as targeted gene knockout studies show that phosphodiesterase 4B (PDE4B) has a crucial role in the cytokine production of monocytes (17–20). A second profile consisted of genes mainly involved in chemotaxis, adhesion, motility, and

TABLE 1
Q-PCR analysis of monocytes of patients with various forms of diabetes compared with healthy control subjects

	Juvenile-onset type 1 diabetes vs. control subjects*		Adult-onset type 1 diabetes vs. control subjects*		LADA vs. control subjects†		Type 2 diabetic vs. control subjects*	
	Fold change (95% CI)†	*P*	Fold change (95% CI)†	*P*	Fold change (95% CI)†	*P*	Fold change (95% CI)†	*P*
n	30		43		30		30	
Inflammation								
PDE4B	0.71 (0.45–1.13)	0.313	1.26 (0.91–1.74)	0.360	2.86 (1.57–5.19)	<0.001	1.32 (0.93–1.87)	0.358
IL6	0.83 (0.28–2.48)	0.977	3.05 (1.34–6.91)	0.013	17.65 (7.42–41.96)	<0.001	6.82 (3.31–14.06)	<0.001
Il1B	0.51 (0.25–1.04)	0.262	2.46 (1.34–4.51)	0.015	10.12 (4.37–23.47)	<0.001	4.66 (2.68–8.10)	<0.001
PTX3	0.95 (0.61–1.48)	0.770	1.79 (1.13–2.84)	0.127	4.69 (2.39–9.19)	<0.001	2.23 (1.59–3.32)	0.003
PTGS2	0.32 (0.18–0.59)	0.011	2.16 (1.31–3.57)	0.006	8.09 (4.30–15.25)	<0.001	3.98 (2.52–6.28)	<0.001
TNF	0.57 (0.32–1.03)	0.216	1.20 (0.68–2.13)	0.627	6.32 (2.98–13.39)	<0.001	2.95 (1.79–4.87)	0.005
TNFAIP3	0.83 (0.52–1.30)	0.619	1.24 (0.81–1.92)	0.502	4.05 (2.34–7.00)	<0.001	1.71 (1.10–2.66)	0.107
HSPA1A	0.60 (0.41–0.88)	0.008	0.84 (0.71–1.00)	0.358	0.95 (0.56–1.59)	0.867	0.83 (0.61–1.13)	0.417
Chemokinesis/motility/adhesion								
CCL7	22.30 (6.61–75.17)	<0.001	6.36 (2.37–17.05)	<0.001	22.16 (6.87–71.48)	<0.001	3.63 (1.30–10.18)	0.018
CCL20	0.80 (0.26–2.48)	0.953	3.00 (1.14–7.87)	0.054	33.17 (9.21–119.44)	<0.001	7.80 (2.94–20.65)	0.002
CXCL2	1.50 (0.77–2.90)	0.270	2.21 (1.20–4.09)	0.028	9.48 (4.00–22.48)	<0.001	4.92 (2.64–9.16)	<0.001
CCL2	4.19 (1.82–9.63)	0.007	2.65 (1.55–4.54)	0.001	4.62 (2.14–9.97)	<0.001	2.53 (1.37–4.67)	0.002
CDC42	1.98 (1.44–2.73)	0.005	1.29 (0.95–1.73)	0.119	2.03 (1.34–3.08)	<0.001	1.44 (1.13–1.84)	0.041
CD9	2.13 (1.32–3.44)	0.116	1.40 (0.98–2.01)	0.047	2.04 (1.28–3.25)	<0.001	2.13 (1.40–3.23)	0.028
STX1A	7.31 (3.61–14.83)	<0.001	1.48 (0.98–2.24)	0.071	2.89 (1.68–4.97)	<0.001	1.72 (1.06–2.77)	0.023
Cell survival/apoptosis								
BCL2A1	1.39 (0.89–2.17)	0.291	1.42 (1.01–1.99)	0.077	3.17 (1.87–5.37)	<0.001	1.95 (1.40–2.72)	0.005
EMP1	2.47 (1.49–4.10)	0.008	1.29 (0.90–1.86)	0.180	3.49 (2.20–5.54)	<0.001	2.13 (1.40–3.23)	0.002
Mapk pathway								
PTPN7	2.52 (1.83–3.49)	<0.001	1.42 (1.07–1.87)	0.038	2.91 (1.82–4.64)	<0.001	1.94 (1.42–2.64)	0.001
DUSP2	1.04 (0.69–1.58)	0.898	2.26 (1.31–3.90)	0.005	7.98 (4.24–15.02)	<0.001	3.43 (2.00–5.88)	<0.001
ATF3	0.88 (0.60–1.29)	0.531	2.02 (1.38–2.96)	0.001	6.07 (3.60–10.25)	<0.001	2.81 (1.98–3.99)	<0.001
NAB2	2.38 (1.32–4.28)	0.025	1.23 (0.77–1.97)	0.366	2.37 (1.23–4.58)	0.006	1.81 (1.09–3.00)	0.043
MAPK6	1.53 (1.09–2.16)	0.102	1.07 (0.83–1.39)	0.624	1.82 (1.04–3.19)	0.005	1.16 (0.79–1.70)	0.394
Metabolism								
FABP5	2.03 (1.25–3.29)	0.019	0.83 (0.65–1.06)	0.504	1.36 (0.74–2.49)	0.158	0.92 (0.59–1.45)	0.934
DHRS3	3.52 (1.60–7.72)	0.001	1.02 (0.75–1.37)	0.694	2.26 (1.43–3.90)	0.001	1.62 (1.08–2.42)	0.031

†The quantitative value obtained from Q-PCR is a cycle threshold (CT). The fold change values between different groups were determined from normalized CT values (CT gene/CT reference gene *ABL*) by the ΔΔCT method ($2^{-\Delta\Delta CT}$, User Bulletin 2; Applied Biosystems, Foster City, CA). Data were standardized to the control subjects (thus, the control subjects were used as the calibrator). The fold change of the control subjects is therefore 1. The same data were also analyzed prior to standardization to the control subject group. These analyses are demonstrated in supplementary Table D. *Values >1, patients have a higher expression than control group; values <1, patients have a lower expression than control group; $n = 59$ control subjects. *P* tested by univariate ANCOVA vs. control subjects; age and sex are included in model.

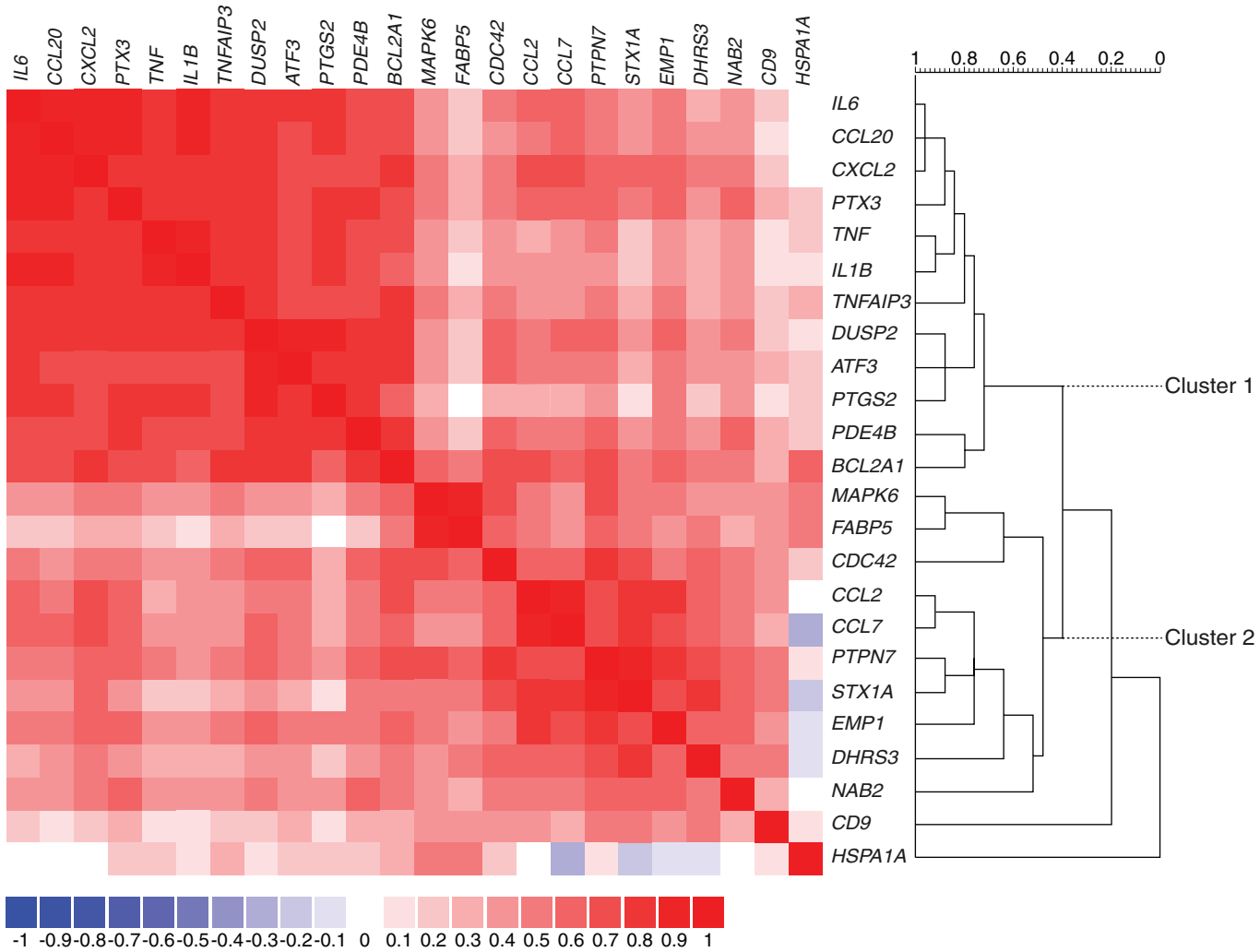

FIG. 1. Color-coded correlation matrix illustrating pairwise correlations between the expression levels of the 24 genes aberrantly expressed in patients with various forms of diabetes (Table 1). Blue squares indicate negative correlations; red squares indicate positive correlations. The color intensities code for the strength of the correlations. Also, a dendrogram is presented as a result of hierarchical cluster analysis with the use of correlation coefficients. The dendrogram shows two gene-expression clusters.

metabolism (*CCL7*, *CCL2*, *CDC42*, *STX1A*, *EMP1*, *FABP5*, *DHRS3*, *NAB2*, *PTPN7*, and *MAPK6*), with a putative key gene *FABP5*.

The first profile (cluster 1) was found in monocytes in LADA (60%) and adult-onset type 1 diabetic patients (28%) more than in juvenile-onset type 1 diabetic patients and control subjects (each 10%). The second profile (cluster 2), conversely, was found in 43% of juvenile-onset type 1 diabetic and in 33% of LADA patients but in <10% each of adult-onset type 1 diabetic patients and control subjects. These different frequencies of the two activation clusters in the known diagnostic categories of diabetes are consistent with the view that the categories are pathologically different, such that LADA and adult-onset type 1 diabetes have similar immune characteristics distinct from juvenile-onset type 1 diabetes (10).

We also found many of the inflammatory genes upregulated in type 2 diabetes monocytes, supporting the view that inflammatory monocytes are involved in the pathogenesis of type 2 diabetes (21). However, most (83–100%) type 2 diabetic patients had normal expression of the key genes *PDE4B* and *FABP5*, which resulted in their monocyte gene cluster being distinct from that in the majority of LADA and juvenile-onset type 1 diabetic patients.

Because the monocytes appear to be distinctly activated and disturbed in LADA, adult-onset type 1 diabetes, juvenile-onset type 1 diabetes, and type 2 diabetes, it is possible that these profiles can be used to identify subforms of diabetes within the known diagnostic categories of diabetes. This subdivision could improve outcome prediction and gene-association studies, may lead to more consistent reports on immune aberrancies in autoimmune diabetes, and could result in new intervention strategies by providing new targets for treatment. PDE4B, in particular, might be such a target, as inhibitors are in development (17–20), and rolipram, an archetypical PDE4 inhibitor, reduced insulitis and prevented diabetes in the nonobese diabetic (NOD) mouse (22). Another potential target for drug intervention is PTGS2, a key enzyme in the biosynthesis of prostanoids. COX-2 (PTGS2) inhibitors are well known for their anti-inflammatory functions (23), but there are no studies of them in diabetic patients. Of note, both a raised and normal basal PTGS2 have been described in type 1 diabetes (3,7); here we find that *PTGS2* is raised in cluster 1 but downregulated in cluster 2–positive type 1 diabetic patients. Thus, COX-2 inhibitors might alter monocyte activation in cluster 1–positive patients (i.e., many LADA and adult-onset type 1 diabetic patients) but

TABLE 2
Correlation of mRNAs

PDE4B correlating set			*FABP5* correlating set		
Gene	*r*	*P*	Gene	*r*	*P*
PDE4B	1		*PDE4B*	0.011	0.96
CCL20	0.88	<0.001	*CCL20*	0.20	0.39
DUSP2	0.88	<0.001	*DUSP2*	0.14	0.45
IL1B	0.87	<0.001	*IL1B*	−0.043	0.96
PTGS2	0.85	<0.001	*PTGS2*	−0.52	0.018
IL6	0.84	<0.001	*IL6*	0.41	0.076
BCL2A1	0.82	<0.001	*BCL2A1*	0.34	0.069
PTX3	0.78	<0.001	*PTX3*	−0.13	0.58
ATF3	0.76	<0.001	*ATF3*	0.21	0.26
TNFAIP3	0.75	<0.001	*TNFAIP3*	0.081	0.67
NAB2	0.69	<0.001	*NAB2*	0.23	0.22
TNF	0.54	<0.001	*TNF*	−0.25	0.19
CXCL2	0.87	<0.001	*CXCL2*	0.60	<0.001
CCL7	0.87	<0.001	*CCL7*	0.66	<0.001
STX1A	0.79	<0.001	*STX1A*	0.68	<0.001
CCL2	0.74	<0.001	*CCL2*	0.73	<0.001
EMP1	0.67	<0.001	*EMP1*	0.57	0.001
CDC42	0.63	<0.001	*CDC42*	0.48	0.008
PTPN7	0.60	0.001	*PTPN7*	0.66	<0.001
MAPK6	0.58	0.001	*MAPK6*	0.50	0.005
DHRS3	0.58	0.001	*DHRS3*	0.66	<0.001
CD9	−0.073	0.70	*CD9*	0.48	0.008
HSPA1A	−0.16	0.41	*HSPA1A*	−0.36	0.005
FABP5	0.27	0.15	*FABP5*	1	

r is Spearman's correlation coefficient. The *PDE4B* correlations were determined in the LADA patients ($n = 30$) because *PDE4B* upregulation was specific for that group. The *FABP5* correlations were determined in the juvenile-onset type 1 diabetic patients ($n = 30$) for the same reason (a specific *FABP5* upregulation in juvenile-onset type 1 diabetes). See Fig. 1, the genes in the red box correlate significantly with *PDE4B*, and the genes in the blue box correlate significantly with *FABP5*.

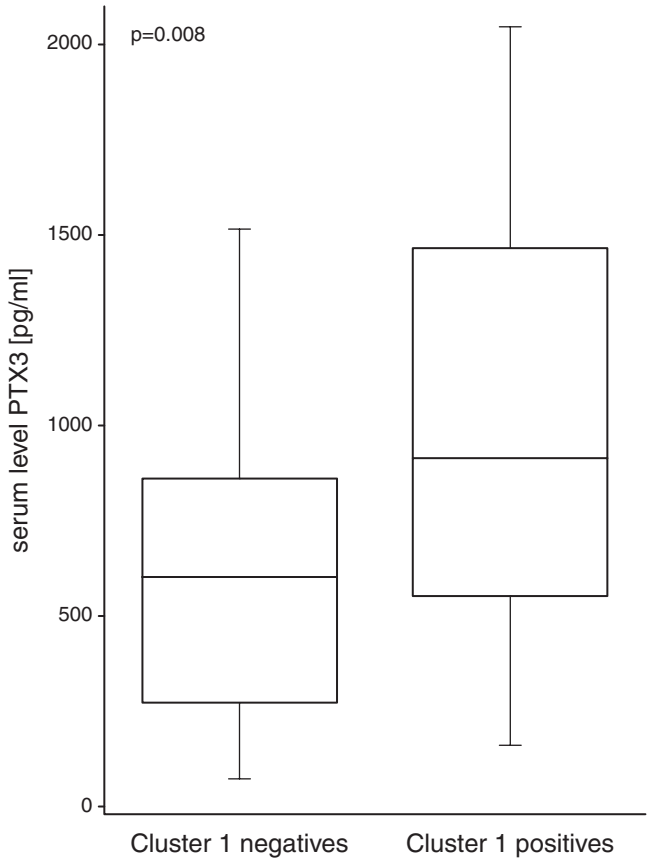

FIG. 2. Serum levels of PTX3 in cluster 1–positive ($n = 36$) and –negative subjects ($n = 73$) (patients as well as control subjects). The definition was as follows: positive, ≥75% of the cluster 1 genes positive; negative, <75% of the cluster 1 genes positive. Groups were compared by ANCOVA analysis with age, sex, and BMI included in the model. Because normal distribution of PTX3 could not be obtained, ranks of PTX3 were used in the analysis (28).

not in cluster 2–positive patients (i.e., many juvenile-onset type 1 diabetic patients).

A recent study (24) showed that factors in serum of type 1 diabetic patients could induce inflammatory genes (*CCL2*, *CCL7*, *IL1B*) in peripheral blood mononuclear cells (PBMCs). Another gene expression study of PBMCs of type 1 diabetic patients (25) also detected overexpression of inflammatory genes (among others, *IL1B* and *PTGS2*), without evidence of the extended signatures described here. In this latter study, investigators used nonfractionated PBMCs. Indeed, an important issue is the cell collection, preservation, and separation used in our study. We used frozen-stored PBMCs and positive CD14 magnetic cell sorting separation. Specifically, freeze-storing might induce differences in gene expression, whereas positive magnetic cell sorting separation does not influence gene expression (11,26). Alternative monocyte separation techniques do modify gene expression profiles, e.g., we found plastic adhered monocytes to downregulate PDE4B gene expression (as is known when monocytes change into macrophages [27]). However, despite these limitations, the differences we describe cannot be due to freeze storage because all monocytes are handled simi-

TABLE 3
The presence of cluster 1 and *PDE4B*–correlating set and cluster 2 and *FABP5*–correlating set in the monocytes of different diabetic groups and control subjects

Definitions	Control subjects	Juvenile-onset type 1 diabetes	Adult-onset type 1 diabetes	LADA	Type 2 diabetes
n	94	30	43	30	30
≥75% of cluster 1 genes positive	10 (9)	10 (3)	28 (12)*	60 (18)†	37 (11)‡
≥75% of PDE4B correlating set genes positive	3 (3)	10 (3)	23 (10)†	43 (13)†	17 (5)‡
≥75% of cluster 2 genes positive	5 (5)	43 (13)‡	9 (4)	33 (10)‡	10 (3)
≥75% of FABP5 correlating set genes positive	2 (2)	43 (13)‡	7 (3)	13 (4)*	0

Data are *n* (%) unless otherwise indicated. For this analysis, 35 extra control subjects were available, so 94 total were studied. Positivity of the genes is defined as an mRNA expression 1 SD higher than the mean level found in the control subjects. *$P < 0.05$; †$P < 0.001$; ‡$P < 0.01$ vs. control subjects (tested via χ^2 tests).

larly. Further investigations are needed to establish consistency and diagnostic and prognostic consequences of monocyte inflammatory profiles under various storage and isolation conditions.

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APPENDIX

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