Peripheral Blood Monocyte Gene Expression Profile Clinically Stratifies Patients With Recent-Onset Type 1 Diabetes

Katharine M. Irvine,1 Patricia Gallego,2,3 Xiaoyu An,1 Shannon E. Best,1 Gethin Thomas,1 Christine Wells,4 Mark Harris,2 Andrew Cotterill,2 and Ranjeny Thomas1

Novel biomarkers of disease progression after type 1 diabetes onset are needed. We profiled peripheral blood (PB) monocyte gene expression in six healthy subjects and 16 children with type 1 diabetes diagnosed 3–9 months previously and analyzed clinical features from diagnosis to 1 year. Monocyte expression profiles clustered into two distinct subgroups, representing mild and severe deviation from healthy control subjects, along the same continuum. Patients with strongly divergent monocyte gene expression had significantly higher insulin dose–adjusted HbA1c levels during the first year, compared with patients with mild deviation. The diabetes-associated expression signature identified multiple perturbations in pathways controlling cellular metabolism and survival, including endoplasmic reticulum and oxidative stress (e.g., induction of HIF1A, DDIT3, DDIT4, and GRP78). Quantitative PCR (qPCR) of a 9-gene panel correlated with glycemic control in 12 additional recent-onset patients. The qPCR signature was also detected in PB from healthy first-degree relatives. A PB gene expression signature correlates with glycemic control in 12 additional recent-onset patients. The versatile cells of the innate immune system, including peripheral blood (PB) monocytes and their differentiated tissue progeny, macrophages, and dendritic cells (DCs), are powerful sensors of stress and danger and mediators of tissue repair. In their role as antigen presenting cells, they orchestrate adaptive immune responses to both endogenous and pathogen-mediated host damage. Paradoxically, innate immune activation may play both pathological and protective roles in type 1 diabetes, depending on disease stage and context. At a molecular level, there is substantial evidence for dysregulated innate immunity in type 1 diabetes (7–10), both before and after disease onset. Activated macrophages and DCs are observed in rodent pancreatic islets before lymphocyte recruitment and are thought to be the primary source of the proinflammatory cytokines tumor necrosis factor (TNF), interleukin (IL)-6, and IL-1β that are implicated in type 1 diabetes pathogenesis (11). Macrophages are also found in islets from recent-onset type 1 diabetic patients (12). In contrast with their proinflammatory roles, macrophages also have immunoregulatory and tissue repair roles, which may be critical in restraining β-cell destruction. Diabetes was exacerbated in mice that lack colony stimulating factor (CSF)-1-dependent pancreatic macrophages, whereas macrophages promoted β-cell survival and function in a CSF-1–dependent manner (13,14).

We hypothesized that PB monocyte phenotype and function constitute sensitive indicators of the prodisease microenvironment, making them ideal biomarkers of innate immune status in type 1 diabetes. Previous PB screening approaches identified proinflammatory signatures, although these studies were in mixed cell populations and were frequently complicated by the proinflammatory effects of hyperglycemia (8,9). We characterized monocyte gene expression in insulin-treated type 1 diabetic patients 3 months after disease onset and analyzed clinical features from diagnosis to 1 year. We report a PB monocyte expression profile, which clinically stratifies glycemic control within the first year after type 1 diabetes diagnosis.

RESEARCH DESIGN AND METHODS

Study population. Sixteen children with recently diagnosed type 1 diabetes (~3 months postdiagnosis; Table 1) were recruited at the Mater Children’s Hospital during 2009 and 2010. Informed consent was obtained from all participants, and the studies were approved by the Mater Health Services and Princess Alexandra Hospital Human Research Ethics Committees. Consenting adult blood donors were used as healthy control subjects for the microarray study (35% female, mean [SD] age 52 years [8.2 years], BMI 24.5 [1.2]), whereas age-matched children without a family history of autoimmune disease recruited from operation list patients awaiting nonurgent surgery served as control subjects.
### Table 1

Characteristics of group A and group B type 1 diabetic patients at diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>9</td>
<td>7</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Female (%)</strong></td>
<td>5 (56)</td>
<td>5 (71)</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years ± SD)</strong></td>
<td>11.5 ± 2.9</td>
<td>11.6 ± 2.8</td>
<td>0.95</td>
</tr>
<tr>
<td><strong>BMI at diagnosis (± SD)</strong></td>
<td>20.2 ± 6.1</td>
<td>20.3 ± 3.1</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Number of patients with ≥1 islet autoantibody (%)</strong></td>
<td>7 (78)</td>
<td>7 (100)</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Mean glucose at diagnosis (mmol/L ± SD)</strong></td>
<td>24.5 ± 6.0</td>
<td>26.4 ± 12.1</td>
<td>0.69</td>
</tr>
<tr>
<td><strong>Mean %HbA1c at diagnosis (± SD)</strong></td>
<td>13.2 ± 0.9</td>
<td>13.4 ± 2.3</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>Mean C-peptide at diagnosis (nmol/L ± SD)</strong></td>
<td>0.19 ± 0.1</td>
<td>0.13 ± 0.03</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Mean 25-OH-vitamin D at diagnosis (nmol/L ± SD)</strong></td>
<td>93.2 ± 37.1</td>
<td>95.0 ± 25.6</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Number of patients presenting with DKA (%)</strong></td>
<td>6 (78)</td>
<td>4 (57)</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Number of patients with high-risk HLADQ genotype</strong></td>
<td>5/7*</td>
<td>3/5*</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Biometrics and clinical parameters recorded at diagnosis of type 1 diabetes. \(P\) values calculated using two-tailed unpaired \(t\) test (discrete variables) or Fisher exact probability test (categorical variables). HCO3\(^{-}\), bicarbonate; DKA, diabetic ketoacidosis. *No genotyping data for 1 of 9 group A and 2 of 7 group B patients. **DQB1*0201, DQB1*0302 and/or DQA1*0501, DQA1*301.

**RESULTS**

**Stratification of type 1 diabetic patients by monocyte expression profile at diagnosis.** Purified PB CD14\(^{+}\) monocytes from a total of 16 recent-onset type 1 diabetic patients and six healthy control subjects were expression profiled using whole genome microarrays (Illumina HT-12), in two separate, independent experiments. Because of the volume of blood required to study purified monocytes, the healthy control subjects comprised young adults. Blood samples for microarray analysis were taken from insulin-treated patients \(\pm 3\) months after type 1 diabetes diagnosis, to avoid the acute metabolic disturbance associated with disease onset. A strikingly similar pattern was observed in both experiments, whereby a subset of monocyte expression profiles clustered apart from the remaining patients and healthy control subjects (group B, Fig. IA and IB). Because of this stark segregation, group B profiles were analyzed separately to identify differentially expressed genes. In group B monocytes compared with healthy control subjects in both microarrays (Fig. 1C and Supplementary Table 2), 1,107 probes (1,015 genes) were at least 1.5-fold differentially expressed (Benjamini Hochberg-corrected \(P\) value < 0.05). Between the entire diabetes cohort and healthy control subjects in both microarrays (Fig. 1D), 320 probes were differentially expressed. Only 38 probes were reproducibly differentially expressed between group A patients and healthy control subjects. Hierarchical clustering of group B–regulated probes revealed that the two putative type 1 diabetes subgroups represent a spectrum of deviation from healthy control subjects, along the same continuum (Fig. 2A and B).

**Extreme divergence from healthy monocyte gene expression correlates with early type 1 diabetes progression.** At diagnosis, group A and group B patients were clinically indistinguishable in terms of HLA genotype, family history, clinical presentation at diagnosis (including symptom duration, ketoacidosis, blood glucose, 25-hydroxyvitamin D, diabetes-associated autoantibodies, or the presence of celiac or thyroid disease; Table 1 and data not shown). Group B patients had higher levels of HbA1c, L and 6

for flow cytometry and peripheral blood mononuclear cell (PBMC) quantitative PCR (qPCR) (mean age [SD] 10 years [4.2 years]). Blood samples were obtained from first-degree relatives (FDR) of type 1 diabetic patients with informed consent (mean age [SD] 9 years [3.9 years]). Insulin dose–adjusted glycosylated hemoglobin (HbA1c) (IDAA1c) was calculated as \(\text{HbA1c} + 4 \times (\text{insulin dose [units/kg/day]})(15)\). Diabetic ketoacidosis (DKA) at presentation was defined as <22 mmol/L plasma bicarbonate.

**Monocyte purification, expression profiling, and qPCR.** CD14\(^{+}\) monocytes were purified from Ficoll-purified PBMC by positive selection (MACS, Miltenyi). Total RNA was purified from CD14\(^{+}\) monocytes using RNeasy Mini columns, with on-column DNase digestion (Qiagen). RNA from the donors selected for microarray (500 ng; Agilent Bioanalyzer RNA Integrity Score >8) was amplified using the Totalprep RNA amplification kit (Ambion). Amplified RNA was hybridized to Illumina HT-12 v3.0 whole genome beadarrays, according to the manufacturer’s instructions. Beadarrays were scanned on an Illumina iScan, and raw data were exported from BeadStudio (Illumina) for further analysis. Gene expression data have been deposited in the gene expression omnibus ( GEO) database (GSE33440, Reviewer Access: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=bfvezxkmsn&db=pubtect&access=GSE33440).}

Data were variance stabilized and quantile normalized using the Lumi package in R (Bioconductor, http://www.bioconductor.org/packages/2.0/bioc/html/lumi.html), followed by clustering and differential expression analysis in GenespringGX (Agilent). Probes that did not achieve an Illumina Detection score of 9 group A and 2 of 7 group B patients. **DQB1*0201, DQB1*0302 and/or DQA1*0501, DQA1*301.

**RESULTS**

**Stratification of type 1 diabetic patients by monocyte expression profile at diagnosis.** Purified PB CD14\(^{+}\) monocytes from a total of 16 recent-onset type 1 diabetic patients and six healthy control subjects were expression profiled using whole genome microarrays (Illumina HT-12), in two separate, independent experiments. Because of the volume of blood required to study purified monocytes, the healthy control subjects comprised young adults. Blood samples for microarray analysis were taken from insulin-treated patients \(\pm 3\) months after type 1 diabetes diagnosis, to avoid the acute metabolic disturbance associated with disease onset. A strikingly similar pattern was observed in both experiments, whereby a subset of monocyte expression profiles clustered apart from the remaining patients and healthy control subjects (group B, Fig. IA and IB). Because of this stark segregation, group B profiles were analyzed separately to identify differentially expressed genes. In group B monocytes compared with healthy control subjects in both microarrays (Fig. 1C and Supplementary Table 2), 1,107 probes (1,015 genes) were at least 1.5-fold differentially expressed (Benjamini Hochberg-corrected \(P\) value < 0.05). Between the entire diabetes cohort and healthy control subjects in both microarrays (Fig. 1D), 320 probes were differentially expressed. Only 38 probes were reproducibly differentially expressed between group A patients and healthy control subjects. Hierarchical clustering of group B–regulated probes revealed that the two putative type 1 diabetes subgroups represent a spectrum of deviation from healthy control subjects, along the same continuum (Fig. 2A and B).

**Extreme divergence from healthy monocyte gene expression correlates with early type 1 diabetes progression.** At diagnosis, group A and group B patients were clinically indistinguishable in terms of HLA genotype, family history, clinical presentation at diagnosis (including symptom duration, ketoacidosis, blood glucose, 25-hydroxyvitamin D, diabetes-associated autoantibodies, or the presence of celiac or thyroid disease; Table 1 and data not shown). Group B patients had higher levels of HbA1c, L and 6

**RESULTS**

**Stratification of type 1 diabetic patients by monocyte expression profile at diagnosis.** Purified PB CD14\(^{+}\) monocytes from a total of 16 recent-onset type 1 diabetic patients and six healthy control subjects were expression profiled using whole genome microarrays (Illumina HT-12), in two separate, independent experiments. Because of the volume of blood required to study purified monocytes, the healthy control subjects comprised young adults. Blood samples for microarray analysis were taken from insulin-treated patients \(\pm 3\) months after type 1 diabetes diagnosis, to avoid the acute metabolic disturbance associated with disease onset. A strikingly similar pattern was observed in both experiments, whereby a subset of monocyte expression profiles clustered apart from the remaining patients and healthy control subjects (group B, Fig. IA and IB). Because of this stark segregation, group B profiles were analyzed separately to identify differentially expressed genes. In group B monocytes compared with healthy control subjects in both microarrays (Fig. 1C and Supplementary Table 2), 1,107 probes (1,015 genes) were at least 1.5-fold differentially expressed (Benjamini Hochberg-corrected \(P\) value < 0.05). Between the entire diabetes cohort and healthy control subjects in both microarrays (Fig. 1D), 320 probes were differentially expressed. Only 38 probes were reproducibly differentially expressed between group A patients and healthy control subjects. Hierarchical clustering of group B–regulated probes revealed that the two putative type 1 diabetes subgroups represent a spectrum of deviation from healthy control subjects, along the same continuum (Fig. 2A and B).

**Extreme divergence from healthy monocyte gene expression correlates with early type 1 diabetes progression.** At diagnosis, group A and group B patients were clinically indistinguishable in terms of HLA genotype, family history, clinical presentation at diagnosis (including symptom duration, ketoacidosis, blood glucose, 25-hydroxyvitamin D, diabetes-associated autoantibodies, or the presence of celiac or thyroid disease; Table 1 and data not shown). Group B patients had higher levels of HbA1c, L and 6
months after diagnosis (Fig. 3A) and required significantly higher insulin doses to maintain glycemic control in the first 12 months after diagnosis (Fig. 3B, P = 0.0004, 2-way ANOVA). Approximately 30% of children diagnosed with type 1 diabetes experience a partial remission phase (honeymoon), during which their HbA1c levels and insulin requirements (used as a proxy for endogenous insulin production from residual β-cells) (15) are relatively low. An insulin dose–adjusted HbA1c metric (IDAA1c) ≥9 correlated with the definition of C-peptide responders in the Diabetes Control and Complications Trial (16) and was proposed as a definition for partial remission. Group A and group B IDAA1c diverged around 9 at 3–6 months postdiagnosis, and group B patients exhibited significantly higher IDAA1c during the first year of disease (Fig. 3C, P < 0.0001; 2-way ANOVA).

To examine whether the monocyte gene expression profiles observed were directly related to hyperglycemia, we identified probes whose expression was significantly correlated (Pearson P < 0.001) with plasma glucose concentration (n = 25), or %HbA1c (n = 70), at the time blood was sampled for profiling. No glucose-correlated probes and 9 of 70 HbA1c-correlated probes were differentially expressed between group B monocytes and healthy control subjects (HC) (n = 1,107) in two independent microarrays were clustered using the Euclidean Distance correlation, as were the experimental samples (A, B).

FIG. 2. Extremely divergent monocyte expression profile in a subgroup of recent-onset type 1 diabetic patients. Genes that were differentially expressed between group B monocytes and healthy control subjects (HC) (n = 1,107) in two independent microarrays were clustered using the Euclidean Distance correlation, as were the experimental samples (A, B).
of tumor necrosis factor (TNF), interleukin (IL)-10, and PTGES2 (which encodes the enzyme catalyzing prostaglandin E2 production), and the prostaglandin E2 receptor, PTGER (Supplementary Table 2 and Fig. 5), whereas multiple genes involved in regulation of immune responses were downregulated in group B monocytes (Fig. 4). These included sensors such as TLR4, TLR5, IFI16, NLRX1, and NLRC4/IPAF and effectors such as CASP1 (Supplementary Table 2). Ten genes in key pathways differentially expressed by group B monocytes were selected for qPCR validation. Their expression was quantified in eight patients profiled previously by microarray and 12 additional recent-onset type 1 diabetic patients. Hierarchical clustering of 9 robustly detected genes segregated group B patients from group A and healthy control subjects, whereas unclassified patients’ monocytes (D) revealed a spectrum of gene expression, similar to the microarray analysis (Fig. 5A). IDAA1c tended to increase with increasing distance from the healthy control subjects expression profile (Fig. 5B; 3 or 6 month linear regression: slope $= -0.225/0.176$, $P = 0.0170/0.091$).

Two genes selected for validation, DDIT4 and adrenomedullin (ADM), were not incorporated in the cluster analysis since they were only detected in monocytes from a subset of type 1 diabetic patients, including group B (Fig. 5C and D).

The group B monocyte expression profile strongly suggested stress-induced metabolic disturbance, with upregulation of ER and oxidative stress responsive genes, and downregulation of genes involved in cellular energy and metabolite production, especially mitochondrial oxidative phosphorylation (e.g., PDHB, MDH1, IDH1, SDHC, ACLY) (Fig. 5 and Supplementary Table 1). Mitochondrion was the most significantly enriched cellular component ontology among group B downregulated genes ($P = 2.6e-05$). It is well established that activation of myeloid and other immune cells is accompanied by a switch in glucose metabolism and cellular energy production, from oxidative phosphorylation to anaerobic fermentation through glycolysis (17). PFKFB3, which encodes the rate-limiting enzyme in the glycolytic pathway, was upregulated in group B monocytes (Fig. 5), but glycolytic pathway genes were not generally induced. PDHB and PFKFB3 expression was strongly inversely correlated in the microarray ($r = -0.86$, $P < 0.0001$), which may imply a reduction in oxidative phosphorylation and increased glycolysis (17). In addition to perturbations in core metabolic pathways, there was also evidence of cellular activation through stress, including the unfolded protein response that is a consequence of ER stress (e.g., IRE1, GRP78, DDIT3, XBP1) (Fig. 5 and Supplementary Table 2). HIF1A, a key mediator of oxidative stress, was upregulated in group B monocytes, as well as multiple HIF1A target genes, including DDIT4, PFKFB3, and ADM (17,18) (Fig. 5 and Supplementary Table 2). Thus, the striking group B monocyte expression profile is a mixed signature consistent with the existence of multiple pathological processes and/or adaptive responses to stress and inflammation.

**Monocyte subset balance is altered in PB of type 1 diabetic patients and a subset of FDR.** The mRNA encoding CX3CR1, a marker of CD16+ monocytes (19), was underexpressed in type 1 diabetic patients compared with healthy control subjects (Fig. 5), suggesting this dysregulation could derive from a perturbation in PB monocyte subset balance. PB monocytes from children with recent-onset or established type 1 diabetic and healthy children were phenotyped in whole blood according to surface expression of CD14 and CD16 (see Supplementary Fig. 2 for representative flow cytometric profiles). We observed a reduction in the total proportion of CD16+ monocytes in PB of type 1 diabetic patients (not shown), which was as a
result of a selective reduction in the proportion of CD14Hi/CD16⁺ monocytes (Fig. 6A). The proportions of CD14Low/CD16⁺ and CD14Hi/CD16⁻ populations were unaltered (Fig. 6B and C), whereas the proportion of CD14Low/CD16⁻ cells was significantly increased in recent-onset type 1 diabetic patients (Fig. 6D). We observed a significant reduction of CD14Hi/CD16⁺ monocytes and an increase in CD14Low/CD16⁻ monocytes in FDR of children with type 1 diabetes, relative to healthy children (Fig. 6E), suggesting that this finding is not associated with hyperglycemia. This phenotype may be monocyte-intrinsic, or may arise because of external sources of stress, which could affect β-cell reserve and PB monocyte subsets in both recent-onset patients and in FDR. The proportion of CD16⁻ monocytes in PB positively correlated with monocyte surface CX3CR1 expression by all CD14⁺ monocytes (Fig. 6F; Pearson's r = 0.4648, P < 0.0001), whereas CX3CR1 surface expression by individual monocyte populations was not altered (data not shown). Thus, the distinctive monocyte signature in recent-onset diabetes, which includes CX3CR1 repression, may partially derive from a relative deficiency in CD14Hi/CD16⁺ monocytes and enrichment in CD14Low/CD16⁻ monocytes.

Subtle changes in cellular composition could underpin differential gene expression in a mixed cell population. We thus investigated whether group B–regulated genes typically exhibited monocyte subset-specific expression patterns. In a meta-analysis of all 16 monocyte microarrays, 98 genes were significantly correlated with CX3CR1 expression (Pearson, P < 0.001). Of the 1,109 genes that were significantly differentially expressed in group B monocytes (|log2| ratio > 1.5 or P < 0.05), 64 were also correlated with CX3CR1 expression (Supplementary Fig. 3). Given the strong correlation between total monocyte CX3CR1 surface expression and %CD16⁻ monocytes in PB, these represent candidate subset-specific genes. We also analyzed two public microarray datasets that compared CD14Hi/CD16⁺, CD14Hi/CD16⁻, and CD14Low/CD16⁻ monocyte gene expression (20,21). There was negligible overlap between the group B monocyte signature and the genes that were differentially expressed between monocyte subsets (data not shown).

ER and oxidative stress markers are upregulated in FDR PBMC. To investigate whether elements of the group B monocyte signature are evident in children at risk of developing type 1 diabetes, we assayed gene expression in
PBMC from FDR, including 14 of 43 with at least one islet autoantibody, compared with healthy children. Purified monocytes were not studied since blood sample volumes were limiting. Broadly expressed genes such as CX3CR1 and HIF1A were not differentially expressed between FDR and healthy control subjects (data not shown). By contrast, increased expression of the inducible ER stress (DDIT3, GRP78) and oxidative stress genes (DDIT4, ADM), and the proinflammatory cytokine TNF, was evident in a subset of FDR (Fig. 7A–E). Expression levels of several of these genes were highly correlated to each other ($P < 0.0001$, Fig. 7F–H and data not shown), confirming our conclusion from the meta-analysis that the stress signature is coincident with, but distinct from, altered monocyte subset distribution in type 1 diabetic patients and FDR.

**DISCUSSION**

There is considerable interest in developing therapies to protect and regenerate residual β-cell function, to arrest or slow the course of disease, and to mitigate the development of type 1 diabetes complications (5). This should be achievable, since 80% juvenile diabetic patients had significant residual β-cell function during the first year after diagnosis (22). However, disappointing results in several recent Phase III immunotherapy trials in recent-onset type 1 diabetes have invited speculation that factors, such as β-cell stress, contribute to continuing β-cell destruction after disease onset and may require treatments other than immune intervention (23).

We report here that PB of children with type 1 diabetes is characterized by a relative deficiency of CD14Hi/CD16+ monocytes, an enrichment in CD14Low/CD162 monocytes, and a striking, coordinated monocyte gene expression reflecting altered metabolism associated with stress. Recent-onset patients could be reproducibly stratified on the basis of their monocyte gene expression profile. Severe deviation from a healthy monocyte expression profile, which we observed in ~50% of patients, independently identified a clinical subset with reduced glycemic control, as evidenced by higher %HbA1c and higher insulin requirement. We hypothesize that the two ends of the spectrum at type 1 diabetes onset represent different disease trajectories, whose differences in glycemic control may affect future development of diabetes complications (1). Identification of individuals on a severe trajectory may provide opportunities for better clinical management or targeted therapies for these patients. The reason for the early clinical divergence in these patients is not clear, but
could be explained by two testable hypotheses: 1) patho-
logical processes are intrinsically more aggressive, or
regulation less effective, in group B patients than group A
patients or 2) group A patients possess the greater β-cell
reserve at diagnosis, and insulin treatment enables at least
transient restoration of function in these cells.

Type 1 diabetes progression is most commonly assessed
using metabolic parameters, including %HbA1c, insulin re-
quirement, and C-peptide levels. C-peptide is the most
common surrogate marker for declining β-cell function,
and thus rate of disease progression. Higher %HbA1c
and insulin requirements were associated with rapid disease
progression in several prospective studies, along with risk
factors such as younger age, presentation with ketoacidosis,
and GAD autoantibody status (4,24,25). Percent HbA1c
levels also predict the development of microvascular com-
plications of type 1 diabetes (26). Type 1 diabetes pro-
gression and remission have rarely been studied from an
immunological perspective, although increased numbers
of regulatory T cells were associated with remission in
one small cohort study (27). It remains to be determined
whether monocyte phenotype represents an independent
biomarker for disease progression or whether monocytes
are simply stress-prone cells that re
fl
ect pancreatic dys-
function. Notably, the mRNA encoding ADM, a vasoactive
hormone involved in protection against oxidative stress
associated with diabetes complications and dysglycemia
(25,28), was upregulated in group B patients and FDR.

Reactive oxygen species (ROS), the causative agents of
oxidative stress, are produced in response to environ-
mental stimuli and as by-products of cellular metabolism.
The distinct oxidative and ER stress signature we ob-
served in group B patients suggests these patients’ mon-
ocyes are intrinsically vulnerable to stress, or that they
exist in a stressful environment. Stress pathway activation
has implications for monocyte differentiation and function.
In addition to the elevated expression of oxidative stress
responsive genes, such as HIF1A and HIF1A targets, mul-
tiple genes involved in cellular antioxidant pathways were
downregulated in group B monocytes (e.g., CAT, G6PD,
OXR1, PRDX1, and PRDX3), suggesting protective mech-
nanisms may be impaired. Oxidative stress is strongly im-
plicated in type 1 diabetes progression, particularly in the
development of complications, in both humans and animal
models (29,30). Oxidative stress is directly associated with
β-cell toxicity and decreased insulin secretion, since
β-cells have intrinsically low antioxidant defenses (29), but
systemic oxidative stress may also play a role in promoting
hyperglycemia and diabetes progression. Monocytes and
neutrophils from type 1 diabetic subjects generated more
ROS (31), and macrophage-derived ROS were found to be
critical for the induction of autoreactive T cells and di-
abetes development in NOD mice (32).

Oxidative and ER stress are closely linked. Correct ER-
dependent protein folding is essential for the localization
and function of secreted and membrane-bound proteins.
FIG. 7. Elements of the type 1 diabetes group B expression signature detected in healthy FDR PBMC. Expression of $DDIT3$ (A), $GRP78$ (B), $DDIT4$ (C), $ADM$ (D), and $TNF$ (E) in PBMCs from type 1 diabetic FDR or healthy children (HC) was quantitated by PCR. Correlation between $DDIT3$ and $GRP78$ expression levels (F) is shown. Correlation between $DDIT4$ and $ADM$ expression levels (G) is shown. Correlation between $TNF$ and $ADM$ expression levels (H) is shown. Correlation between $DDIT3$ expression and %CD14Hi/CD16+ (I) and %CD14Low/CD16+ (J) monocytes in PB is shown. $P$ values for group comparisons (A–D) were generated by Student $t$ test (with Welch’s correction where variances were significantly different, as determined by $F$ test). Pearson’s correlation coefficient ($r$) and associated $P$ values are shown in E–G.
The UPR is a transcriptional program activated in response to a variety of stressors that disturb ER homeostasis, including ROS, infection, lipotoxicity, and impaired calcium homeostasis (33). Although first characterized as a physiological response regulating cell survival under stressful conditions, emerging data demonstrate extensive, bidirectional cross-talk between the UPR, inflammatory, and metabolic pathways (33). For example, ER stress reduced proinflammatory responses in monocytes from type 2 diabetic patients (34) and nonimmune cells (35). By contrast, ER stress has been shown to synergise with inflammatory stimuli in macrophages, specifically enhancing IL-23, IL-8, and IL-6 production, but not affecting IL-12 or IL-1β (36,37). The duration and context of ER stress may thus influence the balance of monocyte inflammatory mediators. Notably, TNF was upregulated in group B monocytes, whereas other inflammatory mediators and signaling molecules were downregulated, including several components of the IL-1β processing pathway that have previously been positively associated with type 1 and type 2 diabetes (38).

Persistent ER stress leads to apoptosis and inflammation, in the event the UPR fails to restore ER homeostasis. The induction of DDIT3, a key UPR proapoptotic effector, in group B monocytes suggests extreme or prolonged ER stress. Because mitochondria are necessary for stress adaptation in the early phase of the UPR, mitochondrial defects should render cells more vulnerable to ER stress–induced death (39). The striking downregulation of mitochondrial component and pathway genes in group B monocytes may affect susceptibility to stress, immune function (40,41), and cellular energy metabolism (42). Genes regulating apoptosis were also enriched in group B monocytes, which may be evidence of a stress-induced challenge to survival in these cells. The detection of stress elements of the group B monocyte signature in unfractio-

mated PBMC from FDR confirms these gene expression changes are not simply a result of hyperglycemia and raises the possibility that oxidative and ER stress also contribute to disease development. Future studies will determine whether the PBMC stress gene expression signature derives solely from monocytes or is common to multiple cell types and will clarify the relationship between monocyte perturbation, diabetes risk status, and disease development.

Monocytes and their differentiated progeny, macrophages and DCs, play both proinflammatory and anti-inflammatory, tissue protective roles. Type 1 diabetic patients and their FDR exhibited a reduced proportion of CD14Hi/CD16– intermediate monocytes in PB and an apparently concomitant increase in the CD14Low/CD16– monocyte subset. Intriguingly, perturbations in PB monocyte subsets did not correlate with the induction of oxidative and ER stress genes in FDR PBMC, suggesting the two phenotypes are independent. Expansion of the CD16– population has been observed in many inflammatory diseas,

es, from infection to autoimmunity (19). A decrease in CD14Hi/CD16– monocytes was associated with cardiovascular events in patients with kidney disease (43,44) and with asthma severity (45). By contrast, increased CD14Hi/CD16– monocyte counts correlated with good clinical outcomes after stroke, potentially because of their roles in tissue repair and angiogenesis (46). CD16– monocytes have also been implicated in promoting immune tolerance (47,48). These two key monocyte functions may be mechanistically linked in type 1 diabetes, since defective clearance of dying cells is also hypothesized to promote inflammation and autoimmunity (49). Wound healing is defective in humans and mice with diabetes, due in part to an inability to efficiently clear apoptotic cells (50). Thus, a relative deficiency in CD16– monocytes may compromise tissue repair and/or autoimmune regulation at sites of inflammation, including the pancreas, which would likely promote disease progression and complications.

In conclusion, we demonstrate that monocyte gene expression distinguishes type 1 diabetic patients at disease onset. This stratification correlates with early disease trajectory, which we hypothesize has implications for the rate of disease progression and the development of complications. The coordinated expression signature in rapidly progressing type 1 diabetes suggests an underlying high-level susceptibility or exposure to physiological stress. Further studies will clarify the impact of the group B monocytes phenotype on antigen-presenting cell function, and thus T cell responses in autoimmune diabetes.

ACKNOWLEDGMENTS

This work was supported by NHMRC Grants 351439 and 569938 and JDRF Grants 1-2006-149 and 25-2010-646. R.T. is supported by Arthritis Queensland and an ARC Future Fellowship.

No potential conflicts of interest relevant to this article were reported.

K.M.I., P.G., M.H., A.C., and R.T. devised the study. K.M.I. analyzed the data, with help from P.G., X.A., M.H., and A.C. K.M.I. wrote the manuscript with input from M.H., A.C., and R.T. K.M.I., X.A., and S.E.B. performed experiments with help from G.T. and C.W. P.G., M.H., and A.C. orchestrated patient recruitment and oversaw clinical analyses and interpretation. A.C. and R.T. provided financial support. R.T. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

The authors thank Tracey Baskerville and Sherrell Cardinal of Mater Health Services for recruiting patients and collecting blood samples.

REFERENCES


3. Bowden SA, Duck MM, Hoffman RP. Young children (<5 yr) and adolescents (>12 yr) with type 1 diabetes mellitus have low rate of partial remission: diabetic ketoacidosis is an important risk factor. Pediatr Diabetes 2008;9:197–201


47. MacDonald KP, Palmer JS, Cronau S, et al. An antibody against the colony-stimulating factor 1 receptor depletes the resident subset of monocytes and tissue- and tumor-associated macrophages but does not inhibit inflammation. Blood 2010;116:3065–3063
Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Irvine, KM; Gallego, P; An, X; Best, SE; Thomas, G; Wells, C; Harris, M; Cotterill, A; Thomas, R

Title:
Peripheral Blood Monocyte Gene Expression Profile Clinically Stratifies Patients With Recent-Onset Type 1 Diabetes

Date:
2012-05-01

Citation:

Persistent Link:
http://hdl.handle.net/11343/259309

File Description:
Published version

License:
CC BY-NC-ND