0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(9):3705–3711

doi: 10.1210/jc.2007-0979

Gene Expression in Peripheral Blood Mononuclear Cells from Children with Diabetes

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Objective: We hypothesized that type 1 diabetes (T1D) is accompanied by changes in gene expression in peripheral blood mononuclear cells due to dysregulation of adaptive and innate immunity, counterregulatory responses to immune dysregulation, insulin deficiency, and hyperglycemia.

Research Design and Methods: Microarray analysis was performed on peripheral blood mononuclear cells from 43 patients with newly diagnosed T1D, 12 patients with newly diagnosed type 2 diabetes (T2D), and 24 healthy controls. One- and 4-month follow-up samples were obtained from 20 of the T1D patients.

Results: Microarray analysis identified 282 genes differing in expression between newly diagnosed T1D patients and controls at a false discovery rate of 0.05. Changes in expression of IL1B, early growth response gene 3, and prostaglandin-endoperoxide synthase 2 resolved within 4 months of insulin therapy and were also observed in T2D, suggesting that they resulted from hyperglycemia. With use of a knowledge base, 81 of 282 genes could be placed within a network of interrelated genes with predicted functions including apoptosis and cell proliferation. IL1B and the MYC oncogene were the most highly connected genes in the network. IL1B was highly overexpressed in both T1D and T2D, whereas MYC was dysregulated only in T1D.

Conclusion: T1D and T2D likely share a final common pathway for β-cell dysfunction that includes secretion of IL-1β and prostaglandins by immune effector cells, exacerbating existing β-cell dysfunction, and causing further hyperglycemia. The results identify several targets for disease-modifying therapy of diabetes and potential biomarkers for monitoring treatment efficacy. **(*J Clin Endocrinol Metab* 92: 3705–3711, 2007)**

TYPE 1 DIABETES (T1D) results from autoimmune destruction of insulin-producing pancreatic β-cells in the Islets of Langerhans (1). This process presumably begins with activation of cellular immunity against self-antigens on β-cells, which likely requires genetic susceptibility combined with environmental insults such as a viral infection. Inflammation then occurs, with invasion of islets by immune effector cells and elaboration of cytokines (2–4). Cytokines such as IL-1β (the product of the IL1B gene) recruit additional inflammatory cells to the islets and have direct cytotoxic effects on β-cells (5). Both inflammation and autoimmune recognition are probably required for efficient destruction of β-cells (6, 7). Diabetes becomes clinically apparent when approximately 90% of β-cell mass has been lost (8).

Developing disease-modifying treatments for T1D requires identification of suitable drug targets and markers of therapeutic efficacy. This requires knowledge of changes in gene expression both in pancreatic β-cells and in immune effector cells. It is difficult to obtain pancreas samples from humans with new-onset T1D because the death rate with proper management is extremely low [~0.1% in our institution (9)]. However, islet-infiltrating immune effectors are presumably in equilibrium with circulating pools and may be sampled in peripheral blood mononuclear cells (PBMCs) (10, 11). Moreover, metabolic derangements associated with diabetes potentially affect all cells in the body, and the resulting changes in gene expression may be sampled in PBMCs. Therefore, we used microarray techniques to identify changes in gene expression in PBMCs from children with new-onset T1D. We observed the time course of resolution of such changes with insulin treatment and determined which of these changes were also found in children with poorly controlled type 2 diabetes (T2D), which is not predominantly an autoimmune disease. These studies identified changes in gene expression in PBMCs that distinguish T1D and T2D as well as changes that are common to both forms of diabetes.

Subjects and Methods

Subjects

The study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Baylor Institute for Immunology Research. Informed consent was obtained from parents or legal guardians, and informed assent was obtained from patients aged 10 yr and older.

Patients between the ages of 2 and 18 yr with newly diagnosed T1D by American Diabetes Association and World Health Organization criteria (12) and healthy controls were eligible. Patients with newly diagnosed T2D as defined by ADA (12) and WHO criteria (13) were required to have hemoglobin A1c (HbA1c) levels of 8% or greater so as to be similar biochemically to the T1D patients. Patients were excluded from the study if they had an active or presumed infection, other autoimmune disease, were pregnant, were taking immune modulators, or had an initial hematocrit less than 27%. Patients were excluded if it was un-

First Published Online June 26, 2007

Abbreviations: C_T, Cycle threshold method; FDR, false discovery rate; GAD65, glutamic acid decarboxylase; HbA1c, hemoglobin A1c; IA-2, insulinoma-associated antigen-2; PBMC, peripheral blood mononuclear cell; SOJIA, juvenile idiopathic arthritis of systemic onset; T1D, type 1 diabetes; T2D, type 2 diabetes.

certain whether they had T1D or T2D. All patients were initially hospitalized for medical management, diabetes teaching, and insulin initiation. Initial samples were obtained after diabetic ketoacidosis (if present) had resolved, within 5 d (but usually within 2–3 d) of diagnosis.

Processing of blood samples

Blood was collected in EDTA tubes. PBMCs were isolated using Ficoll gradients within 4 h of each blood draw; if not processed immediately, cells were lysed in RLT buffer containing β-mercaptoethanol (QIAGEN, Valencia, CA) and stored at −80 C. Total RNA was extracted using RNeasy minikits per the manufacturer's protocol (QIAGEN). RNA integrity was assessed using an Agilent 2100 bioanalyzer (Agilent, Palo Alto, CA).

Autoantibody testing

Serum samples (stored at −80 C) were tested for antibodies to insulin, protein tyrosine phosphatase receptor type N [insulinoma-associated antigen (IA-2)], and glutamic acid decarboxylase (GAD65) using ELISA kits from Kronus Inc. (Boise, ID) at ARUP (Salt Lake City, UT) or in the laboratory of Philip Raskin at University of Texas Southwestern Medical Center.

Flow cytometry

PBMCs from each sample were analyzed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA) using antibodies against CD3, CD14, CD19, and CD16 (Becton Dickinson, Franklin Lakes, NJ) in one well to differentiate between B cells, T cells, monocytes, and natural killer cells. Anti-CD3, -CD14, -CD8, and -CD4 antibodies differentiated between cytotoxic and helper T cells and monocytes. Antilineage fluorescein isothiocyanate cocktail, and anti-CD123, HLA DR, and CD11c antibodies differentiated among various types of dendritic cells, whereas anti-CD27, -CD138, -CD20, and -CD19 antibodies distinguished naïve, memory B cells, and plasma cell precursors. Experiments were analyzed after gating live cells according to forward side scatter/side light scatter. A minimum of 100,000 cells was used for each staining condition, and 5,000–50,000 events were recorded for analysis.

Microarray assays

From 2–5 μg of total RNA, double-stranded cDNA containing the T7-dT(24) promoter sequence was generated using GeneChip one-cycle cDNA synthesis kits (Invitrogen, Santa Clara, CA). This was used as a template for *in vitro* transcription with biotin labels using GeneChip IVT labeling kits (Affymetrix, Santa Clara, CA). Biotinylated cRNA was purified using sample cleanup modules (Affymetrix) and hybridized to human U133A and U133B GeneChips (Affymetrix). Arrays were scanned using a laser confocal scanner (Agilent). Artifacts were masked, and samples with excessive background noise or poor cRNA quality based on internal control genes (actin, or glyceraldehyde-3-phosphate dehydrogenase) were excluded from analysis.

RT-PCR

Two micrograms of cRNA samples were converted to cDNA using TaqMan reverse transcription reagents and a 2720 thermocycler (Applied Biosystems, Foster City, CA). Quantitative real-time PCR was performed in duplicate using 50 ng of cDNA and already-developed TaqMan gene expression assays (Applied Biosystems) on the ABI Prism 7900HT sequence detection system. Data were analyzed (SDS2.3) using the relative comparative cycle threshold method (C_T) with hypoxanthine ribosyl transferase as the endogenous control. Delta C_T values were compared with the negative log of normalized microarray expression data.

Statistical analysis

For each Affymetrix U133A or U133B Gene Chip, raw intensity data were normalized to the mean intensity of all measurements on that chip and scaled to a target intensity value of 500 in GeneChip Operating System version 1.0. With use of Genespring software, version 7.3.1, the value for each gene in each patient sample array was divided by the median of that gene's measurement from the cohort of healthy volunteers. A filter was applied based on Affymetrix flag calls: probe sets were selected if present in at least 50% of samples in any group. Class comparisons expressed as Benjamini-Hochberg false discovery rates (14, 15) were performed using parametric tests (ANOVA) after log transformation.

Results

Study population

We obtained blood samples from 24 healthy volunteers, 43 newly diagnosed T1D patients, and 12 newly diagnosed T2D patients (Table 1). We collected samples 1 and 4 months after diagnosis from the last 20 of the T1D patients. For each time point, one sample did not pass quality control and was dropped from the analysis. Patients with T2D were distinguished from T1D on the basis of age, body habitus, presence (11 of 12 patients) of acanthosis nigricans, family history of T2D (11 of 12 patients), and absence of autoantibodies to insulin, IA-2, and GAD65. We allowed low titers of insulin antibodies in T2D patients (≤4 U/ml), which have been previously reported (16–18). All but two of the T1D patients with positive antiinsulin antibodies were also positive for at least one additional autoantibody. One teenager with putative T1D was excluded from the study because he was negative for all three antibodies. One putative T2D patient was excluded when she was found to be positive for IA-2 and GAD.

Flow cytometric results

We found no statistically significant differences between healthy controls and subjects with newly diagnosed T1D in the absolute number of CD14+ monocytes, CD123+ and CD11c+ dendritic cells, basophils, T cells of CD4+/3+, CD8+/3+, or CD8+/3− phenotypes, CD20+/27− naïve B cells, or CD19+/14− B cells. Plasma cell precursors (CD19+/20−) were increased ($P = 0.02$) in new-onset T1D patients but not T2D patients; however, this was not statistically significant after correcting for multiple comparisons. One month after T1D diagnosis, the absolute number of plasma cells was not statistically different from that of healthy controls.

TABLE 1. Study demographics

	Healthy controls (n = 24)	T1D (n = 43)	T2D (n = 12)
Age, yr (mean ± SD)	11.3 ± 4.6	10.1 ± 3.8	14.0 ± 2.3
Sex (% female)	58	60	58
Race	11 Caucasian 7 Hispanic 6 Mixed or unknown ethnicities	28 Caucasian 7 Hispanic 3 African-American 4 Mixed ethnicities	2 Caucasian 2 Hispanic 7 African-American 1 Asian
Body mass index (mean Z score ± SD)	Unknown	0.03 ± 1.33	2.33 ± 0.32
Initial pH less than 7.3	n/a	37%	17%
Initial HbA1c (mean ± SD)	n/a	11.8 ± 2.0	12.2 ± 1.5

Microarray results

Of the 44,760 probe sets on the Affymetrix microarrays, 21,514 had flag calls of present. At a false discovery rate (FDR) of 0.05 (corresponding to an uncorrected *P* value of 0.00072 in this data set), 312 probe sets representing 282 unique genes differed in expression between new-onset T1D patients and healthy controls (supplemental table, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org). An FDR of 0.01 yielded 51 probe sets representing 49 unique genes, and 23 probe sets (22 genes) differed at the stringent Bonferroni-corrected *P* value of 0.05 (Fig. 1). The most overexpressed genes in T1D patients were IL1B, early growth response genes (EGR)-2 and EGR3, prostaglandin-endoperoxide synthase 2 [PTGS2; cyclooxygenase-2], chemokine (C-C motif) receptor 1 (CCR1), and the FOSB oncogene; their expression was increased 2- to 9-fold over healthy controls. The most significantly underexpressed genes (1.5- to 3-fold) included RAB12 (a member of the RAS oncogene family), splicing factor, arginine/serine-rich 15, N-glycanase, and solute carrier 25A29.

To confirm these results using a different statistical approach, we compared the initial 23 newly diagnosed T1D patients to the healthy controls, identifying the probe sets that differed between these groups at an FDR of 0.05 using nonparametric Mann-Whitney tests. Confining the analysis to these probe sets, we then compared samples from the 20 subsequent T1D patients to the same group of healthy volunteers using an FDR of 0.05. This statistical comparison generated a list of 130 unique genes, which included 39 of 49 of the differentially expressed genes with an FDR of 0.01 from the first analysis and 18 of 22 of the genes that differed at a Bonferroni-corrected *P* value of 0.05 (not shown).

We compared the expression of the most differentially expressed genes at baseline to 1 and 4 months after diagnosis. Even with improvement in overall glycemic control (average initial HbA1c level of 11.8 ± 2.0% decreased to 7.1 ± 1.3% by 4 months), EGR2 remained overexpressed in patients ($P = 0.0006$ *vs.* healthy controls) at 4 months after diagnosis, whereas EGR3, IL1B, CCR1, and FOSB decreased toward healthy control levels (Fig. 2). RAB12, splicing factor, arginine/serine-rich 15, NGLY1, and solute carrier 25A29 remained underexpressed throughout the study period.

Eighteen of the 22 most highly differentially expressed genes in newly diagnosed T1D were similarly regulated in T2D (Fig. 1).

Genes specifically expressed in plasma cells (such as Ig genes) were generally more highly expressed in T1D patients than controls or T2D patients; of 76 genes associated with plasma cells (Chaussabel, D., C. Glaser, J. Shen, P. Patel, N. Baldwin, D. Stichweh, L. Bennett, L. Monnet, W. Allman, F. Allantaz, *et al.*, unpublished observations), 57 (75%) were overexpressed with uncorrected $P < 0.05$. Mean expression for the 76 genes was correlated with a Spearman r of 0.53 (95% confidence interval, 0.30–0.71; $P < 0.0001$) to absolute plasma cell numbers determined from flow cytometry. There was no correlation between the number or titer of positive autoantibodies and number of plasma cells.

RT-PCR

To confirm selected microarray results using an independent technique, normalized microarray values were compared with delta C_T values for the same genes obtained from RT-PCR experiments. Spearman r values ranged from 0.62 to 0.94 for six genes (Fig. 3) with *P* values ranging from 0.0031 to less than 0.0001.

Pathway analysis

To identify functional relationships between differentially expressed genes, we used a predefined knowledge base containing more than 10,000 curated human genes (19) (Ingenuity Systems, Redwood City, CA). Of the 21,514 defined as present on the arrays, 5897 genes had entries within the knowledge base. When an FDR of 0.05 was used as a threshold criterion (282 genes differentially expressed between new T1D patients

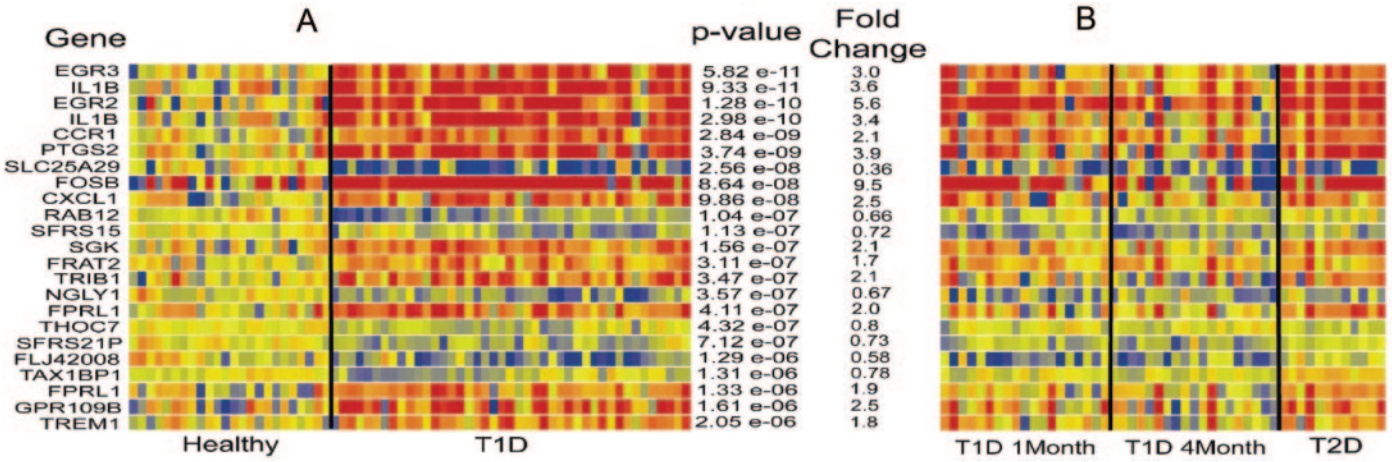

FIG. 1. A, Heat map representing 23 gene probes differentially expressed with a Bonferroni-corrected $P < 0.05$ when comparing newly diagnosed T1D patients with healthy controls. Each row represents a separate probe set, and each column a separate patient sample. IL1B is represented by two probe sets. Each pixel is colored from *red* (5-fold overexpressed) through *yellow* (equal) to *blue* (5-fold underexpressed), compared with median of healthy controls. The uncorrected *P* value for each comparison and the fold change (median) are listed to the right of the panel. B, Expression levels of the same gene probes are illustrated in T1D patients at 1 and 4 months after diagnosis and in T2D patients.

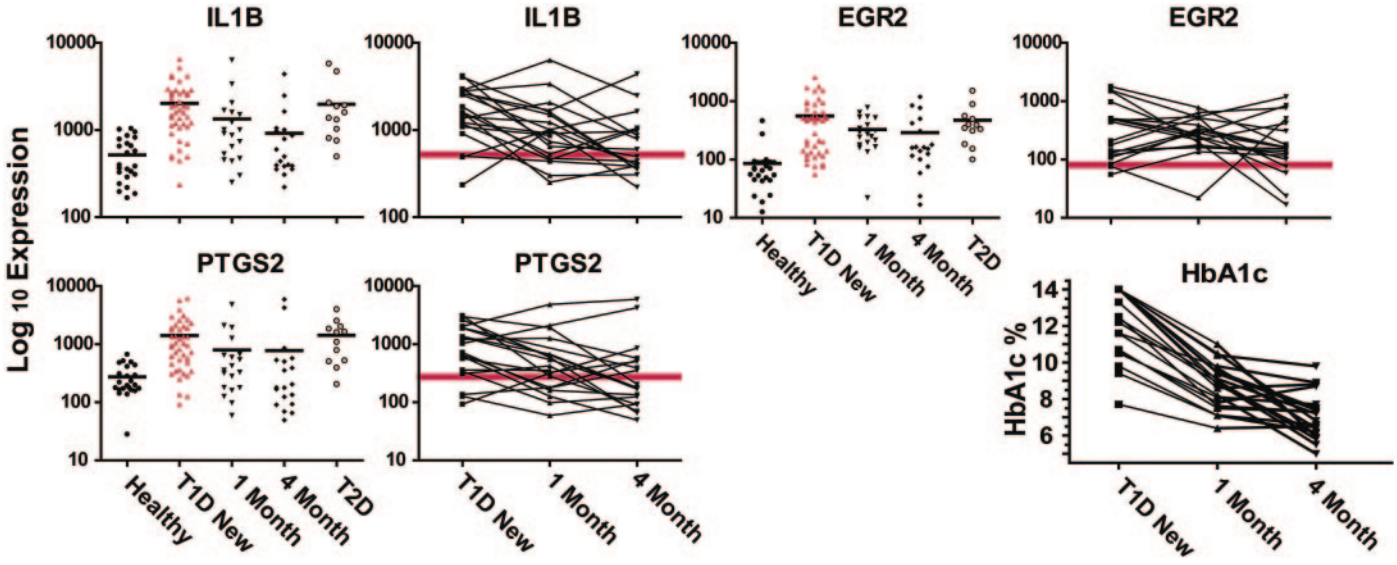

FIG. 2. Levels of expression of selected genes in healthy controls, T1D patients at diagnosis (new) and 1 and 4 months later, and T2D patients. The scale is logarithmic. Individual data points and means are displayed. New T1D and T2D patients have similar expression levels of IL1B, PTGS2, and EGR2. The expression plots depict changes in expression of the genes over time for individual T1D patients. The *horizontal lines* represent mean expression level in healthy controls. Levels of expression do not decrease in parallel with HbA1c levels.

and healthy controls), 11 partially overlapping subnetworks were enriched for these genes. The top-scoring subnetwork included 35 genes meeting the threshold criterion with a probability of 10^{-61} (Fisher's exact test) that the interrelationships between these genes occurred by chance. This network was extended by merging all overlapping networks. Genes within these networks that did not meet the threshold FDR of 0.05 were retained if they were differentially expressed with an uncorrected P value of 0.05. The resulting network of 103 genes had a probability score of 10^{-93} and preferentially included the most differentially expressed genes: 81 of 282 genes in the input data set that differed at an FDR of 0.05 were included in this network *vs.* 11 of 22 genes that differed at a Bonferroni-corrected P value of 0.05 ($P = 0.033$ by Fisher's exact test). There were 222 connections (*i.e.* known relationships) between genes in this network (Fig. 4).

To identify groups of differentially expressed network genes unique to T1D, we compared levels of expression in T1D with those in T2D, identifying 47 of 103 genes that differed between T1D and T2D at an FDR of 0.05. These genes tended not to be distributed randomly within the network, as illustrated by considering the two most highly connected genes in the network, IL1B and MYC (36 connections each). IL1B is similarly overexpressed in T1D and T2D patients. In contrast, MYC is overexpressed only in T1D patients; thus, it differs significantly in expression between T1D and T2D patients. When the 10 genes that are connected in the network to both IL1B and MYC were excluded, 16 of 26 genes connected to MYC, but only nine of 26 genes connected to IL1B, differed in expression between T1D and T2D ($P = 0.05$, Fisher's exact test).

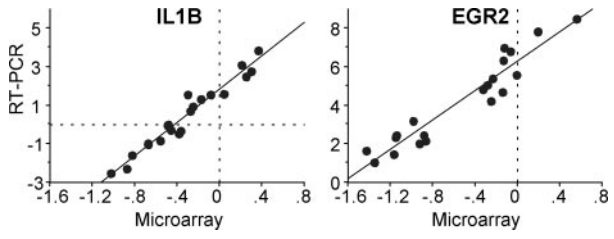

FIG. 3. RT-PCR results of EGR2 and IL1B were correlated to Genespring-generated results for 14 T1D, seven healthy, and three T2D patients using delta C_T results of RT-PCR and the negative logarithm of normalized Genespring values. Spearman r values were: EGR2, 0.91; IL-1B, 0.94 ($P < 0.0001$ for both); EGR3, 0.77; FOSB, 0.61; PTGS2, 0.82; SGK, 0.73 (graphs not shown).

The cellular functions most strongly associated with this network (Table 2) include cell death (51 genes, $P < 5 \times 10^{-18}$) and cell proliferation (50 genes, $P < 10^{-13}$). Excluding genes connected to both IL1B and MYC, genes connected to IL1B were more likely to have functions associated with proliferation (19 of 26) than genes connected to MYC (seven of 26, $P = 0.002$, Fisher's exact test) whereas genes associated with apoptosis were equally likely to be connected to IL1B or MYC (14 of 26 *vs.* 12 of 26, respectively).

Discussion

With more than 40,000 probe sets, whole-genome microarray studies are susceptible to type I errors because they simultaneously test multiple hypotheses. The most frequently used method of controlling the type I error rate and maintaining adequate power (controlling the type II error rate) is the FDR (14, 15), the expected proportion of truly null hypotheses among all the rejected null hypotheses. In some studies, this is balanced by concurrent consideration of false-negative rates (20).

A powerful alternative strategy consists of testing for differences in expression of predefined clusters or networks of genes rather than individual genes, thus reducing the number of tested hypotheses. We used such an approach to delineate similarities and differences in gene expression between T1D and T2D patients. Most (51 of 81) of the

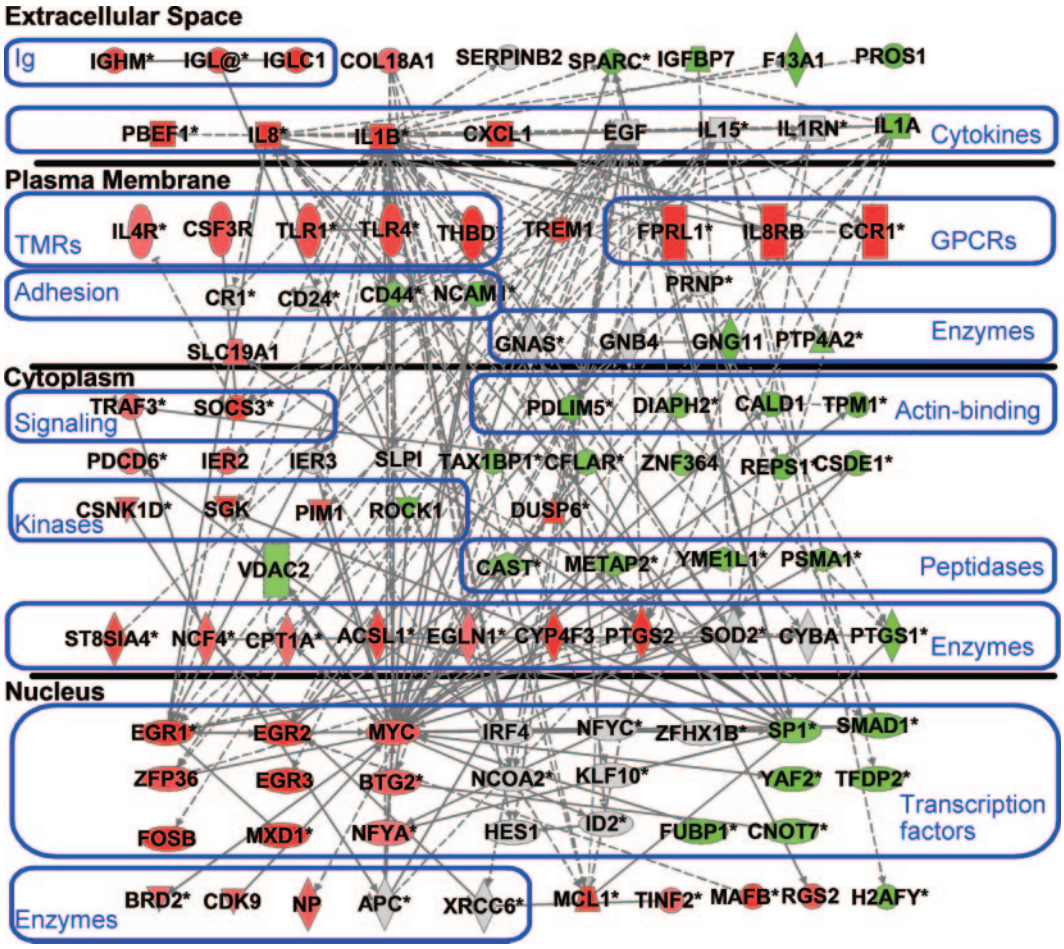

FIG. 4. Network of genes with altered expression in T1D. *Solid lines* represent proteins that are known to physically interact, whereas *broken lines* denote indirect relationships. *Red and green objects* denote genes that are overexpressed or underexpressed, respectively, in T1D patients at diagnosis, relative to healthy volunteers. *Gray genes* differ in expression levels between T1D patients and healthy volunteers at uncorrected $P < 0.05$ but not at FDRs less than 0.05. Genes are positioned to represent their function and site of action within a cell. TMRs, Transmembrane receptors; GPCRs, G protein-coupled receptors.

differentially expressed genes in the network have no prior reported associations with diabetes, diabetes complications, or hyperglycemia.

Our data suggest that T1D and T2D have some pathogenic mechanisms in common (probably due to hyperglycemia and exemplified by overexpression of IL1B, as previously proposed) (2) despite their distinct underlying etiologies (evidenced by overexpression of MYC only in T1D patients).

TABLE 2. Cellular functions associated with T1D based on Ingenuity pathways

Function	*P* value	No. of genes
Apoptosis of eukaryotic cells	4.74E-18	51
Proliferation of cells	9.52E-14	50
Development of lymphatic system cells	2.05E-13	20
Quantity of cells	1.28E-12	36
Cell death of tumor cell lines	1.37E-12	34
Hematopoiesis	1.60E-12	25
Quantity of lymphatic system cells	3.48E-10	22
Quantity of leukocytes	5.42E-10	21
Production of prostaglandin E2	1.90E-9	9
Inflammatory response	2.72E-9	19

Gene expression changes common to T1D and T2D

IL-1β has previously been implicated in the pathogenesis of diabetes (21). Patients with diabetes are hyperglycemic at diagnosis; IL-1β is induced in monocytes *in vitro* by high glucose levels (22). Incubation of human or animal islets or insulinoma cell lines with IL-1β (along with TNFα and/or interferon-γ in many experiments) inhibits insulin secretion and leads to apoptosis of β-cells (23). Of genes connected to IL1B in the network, the most evidence for dysregulation in diabetes exists for PTGS2 (cyclooxygenase-2), which is increased in mononuclear cells from established diabetic patients (22, 24) and is also up-regulated *in vitro* by high glucose concentrations (22).

It is instructive to compare diabetes with a disease in which IL-1β is known to play a pathogenic role, juvenile idiopathic arthritis of systemic onset (SOJIA). There is a median 1.7-fold increase in IL1B expression in SOJIA PBMCs *vs.* healthy controls (25), compared with a greater than 3-fold median increase in newly diagnosed T1D patients. Of the top 10 mostly highly overexpressed genes in T1D patients, five, IL1B, EGR3, PTGS2, CCR1 and CXCL1, are also overex-

pressed in SOJIA patients and/or are overexpressed when healthy PBMCs are incubated with SOJIA serum (25). Although our data suggest the importance of IL-1B dysregulation in diabetes as well as SOJIA, diabetes is not the sole result of IL-1β secretion because patients with diabetes do not have systemic effects of IL-1β-mediated inflammation such as fever and arthritis. Conversely, although IL1B is up-regulated in both diabetes and SOJIA, it is not overexpressed in all diseases that affect the immune system. It is not overexpressed in patients infected with influenza A (26), patients with systemic lupus erythematosus (27), or in recipients of liver transplants (Chaussabel, D., C. Glaser, J. Shen, P. Patel, N. Baldwin, D. Stichweh, L. Bennett, L. Monnet, W. Allman, F. Allantaz, *et al.*, unpublished observations).

It has been suggested that T1D and T2D share a final common pathway for β-cell dysfunction: hyperglycemia in pancreatic islets up-regulates IL1B, leading to β-cell dysfunction and further hyperglycemia (2, 28). However, hyperglycemia has not been consistently documented to affect IL-1β secretion by β-cells (29). Data from this study and others (30, 31) suggest that this conundrum may be resolved by postulating that hyperglycemia-induced β-cell dysfunction is mediated in part by immune effectors such as macrophages: β-cell dysfunction leads to hyperglycemia, increasing inflammation (including secretion of IL-1β and prostaglandins by immune effector cells), thus exacerbating β-cell dysfunction, and causing more hyperglycemia

The mechanisms by which hyperglycemia increases IL1B expression in PBMCs remain to be determined. Perhaps protein glycation resulting from chronic hyperglycemia increases IL-1β levels. Advanced glycation end products interact with the receptor for advanced glycation end products and trigger release of IL-1β from monocytes in some (31) but not all studies (32). The involvement of relatively long-lived advanced glycation end products could explain why many of the changes in the present study persisted for several months after insulin treatment was initiated despite improvement in glycemic control.

Changes in gene expression specific for T1D

Although dysregulation of MYC has not been previously reported in human diabetes, it is overexpressed in peripheral leukocytes of diabetes-prone nonobese diabetic mice, relative to control C57BL6 mice, before development of diabetes (33). Transgenic mice in which MYC is overexpressed in pancreatic β-cells develop neonatal diabetes with increased islet hyperplasia accompanied by a marked increase in apoptosis and decreased insulin gene expression (34). The present results extend these findings by demonstrating increased expression of MYC in peripheral leukocytes at diagnosis of T1D and associated dysregulation of many genes implicated in apoptosis. Some of these changes are not seen in T2D patients with similar levels of hyperglycemia but persist for at least 4 months after T1D diagnosis. Therefore, changes in expression of MYC and associated genes are not a simple response to hyperglycemia. Whether the changes affect quantity or functioning of immune effectors, or reflect correspondingly dysregulated gene expression within pancreatic β-cells cannot yet be determined.

Study limitations

The present study design has inherent limitations. First, we sampled PBMCs rather than pancreatic islets. Although islet-infiltrating immune cells are presumably in equilibrium with circulating pools (10, 11), they are diluted in the circulation. Similarly, changes in gene expression that are confined to a particular cell type may be difficult to detect in unfractionated PBMCs (35). Nevertheless, PBMCs reflect generalized abnormalities in immune regulation as well as systemic effects of the metabolic derangements of untreated diabetes. We hypothesize that many of the observed changes are directly or indirectly the consequence of chronic hyperglycemia. Whereas many such changes may be accompanied by parallel changes in pancreatic β-cells, it will be difficult to definitively answer this question due to the inaccessibility of the pancreas in newly diagnosed T1D patients.

Second, the Ingenuity knowledge base, although extensive, is incomplete with regard to interrelationships between genes (*i.e.* the analysis is subject to literature biases), and conversely, many of those relationships are of uncertain functional significance or may be irrelevant in PBMCs.

Third, we studied patients with new-onset diabetes. Key events may have run their course by the time hyperglycemia supervenes. We found no evidence of interferon-γ or TNF-α overexpression in PBMCs from newly diagnosed T1D patients, yet many studies implicate both of these cytokines in diabetes pathogenesis. Perhaps they are involved in human T1D earlier in the course of the disease, but differences between animal models of T1D and humans might also account for this discrepancy.

Therapeutic implications

Although abnormalities in PBMCs in new-onset T1D patients become less prominent over the first few months of insulin therapy, further damage to β-cells is occurring during this time. Many of the observed changes in gene expression resolve with insulin therapy, which provides another rationale for the beneficial effects of aggressive glycemic control early in the disease (36). Our results suggest several promising therapeutic targets. Elevated expression of PTGS2 (and, presumably, high prostaglandin levels) could be treated with nonsteroidal antiinflammatory agents; sodium salicylate was first suggested as a treatment for diabetes in the 19th century (37). The elevation in IL1B expression could be treated with anakinra (IL-1 receptor antagonist protein), which has proven highly effective in SOJIA (25) and familial autoinflammatory diseases (38). Moreover, a 13-wk course of anakinra has recently been reported to improve glycemic control in T2D patients (39). Given that T1D and T2D have very similar dysregulation of IL1B in our data set, it is likely that anakinra will be efficacious, at least over the short term, in improving glycemic control in T1D patients. Blockers of chemokine receptors including CCR1 have reached phase 2 clinical trials as antiinflammatory agents (40). In addition to providing rationales for therapeutic interventions, abnormalities detected in the present study might ultimately provide useful biomarkers for the efficacy of disease-modifying interventions.

Acknowledgments

We thank the physicians and nurses of Children's Medical Center Dallas who assisted in recruiting patients for the study and the patients and their families for their participation. We thank Vicky Cantrell, Minning Wu, and Zhongbo Jin for technical support and Florence Frager for helpful discussions.

Received May 1, 2007. Accepted June 14, 2007.

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This work was supported by the Children's Medical Center Foundation, Baylor Research Foundation, Mary Celestia Fisher Fund, and Grants U19AIO57234, P50AIO54083, and T32DK007307 from the National Institutes of Health. P.C.W. holds the Audre Newman Rapoport Distinguished Chair in Pediatric Endocrinology at the University of Texas Southwestern Medical Center. J.B. holds the Caruth Chair in Transplantation at Baylor Institute for Immunology Research.

Disclosures: The authors have nothing to disclose.

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