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Immune profiling by multiple gene expression analysis in patients at-risk and with type 1 diabetes

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Abstract There is a need for biomarkers to monitor the development and progression of type 1 DM. We analyzed mRNA expression levels for granzyme B, perforin, fas ligand, TNF-α, IFN-γ, Foxp3, IL-10, TGF-β, IL-4, IL-6, IL-17, Activation-induced cytidine deaminase (AID) and Immunoglobulin G gamma chain (IgG<gamma>) genes in peripheral blood of at-risk, new-onset and long-term type 1 DM , and healthy controls. The majority of the genes were suppressed in long-term type 1 DM compared to controls and new-onset patients. IFN-γ, IL-4 and IL-10 mRNA levels were significantly higher in new-onset compared to at-risk and long-term groups. There was decreased mRNA expression for AID and IgG<gamma> and up-regulation of IFN-γ with age in controls. Data suggest an overall depressed immunity in long-term type 1 DM. Increased gene expression levels for IFN-γ, IL-4 and IL-10 in new-onset patients from at-risk patients might be used as potential markers for progression of the disease.

Abbreviations: AID, activation-induced cytidine deaminase; FasL, fas ligand; FDR, false discovery rate; IgG<gamma>, immunoglobulin G gamma chain; NOD, non-obese diabetic; Type 1 DM, type 1 diabetes mellitus.

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1. Introduction

Type 1 diabetes mellitus (type 1 DM) results from cell-mediated autoimmune destruction of insulin-producing pancreatic beta cells. The activation of autoimmune responses is believed to be initiated by antigen presenting cells [1]. Following activation, CD4 and CD8 T cells, along with B cells, participate in the destruction of beta cells [2–5]. T cell-mediated beta cell damage involves the release of cytotoxic molecules which include cytokines (e.g., TNF-α, IL-1β), granzyme B, perforin, and the Fas–Fas ligand interaction [6,7]. Activation of the caspase pathway by these molecules leads to apoptosis of the beta cell. Hence, antigen presenting cells, T cells (CD4+ and CD8+), and cytokines interact to destroy beta cells [8].

Proinflammatory cytokines, such as IFN-γ and TNF-α, play a role in initiation of early inflammatory processes [9] and seem to promote beta-cell destruction [10,11] while regulatory cytokines, such as IL-10 and TGF-β, seem to regulate beta-cell destruction [7,8,13]. IFN-γ has been observed in islets in vivo in patients with new-onset type 1 DM [12]. Studies of the peripheral immune system of patients with new-onset type 1 DM have shown significantly higher levels of IL-1α, IFN-γ, and TNF-α as compared to healthy controls [13,14]. Th17 cells have been shown to play a crucial role in the induction of autoimmune tissue injury, inflammation, and infection and may be involved in exacerbation of diabetes [15,16]. Increased IL-17 secreting T cells were reported in children with new-onset type 1 DM [17]. B cells have also been implicated in type 1 DM development [3], but the actual role that B cells play is not yet clear. Data from a recent clinical trial demonstrated that targeting B cells in new-onset type 1 DM patients with anti-CD20 (Rituxan) preserved residual insulin secretion for at least 1 year [18]. A recent study on samples from pancreas from new-onset type 1 DM patients showed that CD20+ cells were present in only small numbers in early insulitis, but were recruited to islets as beta cell death progressed, suggesting an increasing role for these cells as insulitis develops [4]. Activation-induced cytidine deaminase (AID) is a molecule selectively expressed in vivo and in vitro in class switch recombination-induced B cells [19] and was used here because our previous work showed it (and immunoglobulin IgG) to be a good biomarker for human B cell responses [20]. There is reported evidence for linkage and association of type 1 DM with genetic markers located at the immunoglobulin heavy chain gene cluster [21], thereby showing a possible role of immunoglobulin heavy chain in the pathogenesis of type 1 DM.

The cytotoxic lymphocyte gene products granzyme B, perforin, and fas ligand (FasL) have been shown to play an integral part in the development of type 1 DM [22]. We previously reported that mRNA levels of perforin and FasL genes were significantly lower in patients with long-term (>5 years) type 1 DM as compared to healthy controls [23]. There have been other studies reporting similar findings regarding Fas function. Defective expression of the Fas molecule on T and B lymphocytes was reported in patients with both newly diagnosed and long-term diabetes [24]; patients with other autoimmune diseases were also found to have defective Fas function [25]. Detection of insulin gene expression in peripheral blood for monitoring the release of β cells was reported to be effective as a marker of injury to the islet graft after transplantation [26]. We included the insulin gene in this study to find out if β cell injury might be evident in patients in different stages of type 1 DM development.

There have been conflicting reports about the levels of cytokine secretion and cytokine mRNA expression in at-risk, new-onset, and long-term type 1 DM patients [27,28]. These discrepancies could be due to different stages of the disease even within the same group [29]. Some studies hypothesized that an active Th1-like immune response destroys beta cells, followed by presentation of autoantigens during the prediabetic phase [29,30].

Gene expression profiles in peripheral blood or in a specific blood cell population may provide new insights into the pathogenesis of type 1 DM [31–33]. Such studies could reveal differences in immune responsiveness between patients with type 1 DM and healthy controls and may identify changes in gene expression that associate with progression of at-risk patients to new-onset type 1 DM. To this end we measured expression levels for fourteen candidate genes, in the peripheral blood of at-risk, new-onset and long-term type 1 DM patients and healthy subjects, including: insulin, cytotoxic lymphocyte genes granzyme B, perforin, and FasL, and some key T and B cell immune mediators, including TNF-α, IFN-γ, Foxp3, IL-10, TGF-β, IL-4, IL-6, IL-17, AID, and immunoglobulin G gamma chain (IgG<gamma>).

2. Methods

2.1. Subjects

Peripheral blood from four groups (*n*, age ± SD in years, male/female ratio): at-risk (*n* = 18, 14.8 ± 12.4, 10/8), new-onset (*n* = 29, 15.3 ± 8.2, 15/14), and long-term (*n* = 62, 39.3 ± 13.8, 32/30) type 1 DM patients and healthy controls (*n* = 80, 39.0 ± 10.2, 39/41) were included in the study. At-risk and new-onset patients were selected from those patients undergoing screening for enrollment in Type 1 Diabetes TrialNet studies at the University of Miami. At-risk patients have at least one confirmed positive autoantibody: insulin (IAA), glutamic acid decarboxylase-65 (GAD65), ICA512/IA2 (IA2), and/or ICA. These autoantibodies were measured in the Type 1 Diabetes TrialNet core laboratories. Risk status of the at-risk patients were stratified based on the protocol for Type 1 Diabetes TrialNet Natural History Study [34]: low risk (*n* = 2, defined as having one positive autoantibody with a normal oral glucose tolerance test), moderate risk (*n* = 10, defined as having two positive autoantibodies with a normal oral glucose tolerance test), and high risk (*n* = 6, defined as having three or more positive autoantibodies with a normal oral glucose tolerance test or 1–4 positive autoantibodies with an abnormal oral glucose tolerance test). Diagnosis of diabetes was made according to guidelines from the American Diabetes Association [35] and all patients in the new-onset group were diagnosed within a year of sample collection with the mean disease duration of 80 ± 84 days. Long-term (>5 years duration, mean disease duration = 22.8 ± 11.5 years, average HbA1c (%) = 7.8 ± 1.3) type 1 DM patients were identified from those undergoing screening for studies in clinical islet transplant trials at the University of Miami. Control blood samples were obtained from healthy volunteers at the University of Miami (*n* = 60) and at Beckman-Coulter, Inc. (Miami, FL) (*n* = 20). No