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## **T-helper Cell Type-1 Transcription Factor T-Bet Is Down-regulated in Type 1 Diabetes**

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### **ABSTRACT**

T cells have been identified as key players in the pathogenesis of type 1 diabetes. However, the exact role of T-cell subpopulations in this pathway is presently unknown. The purpose of this study was to assess the expression pattern of two lineage-specifying transcription factors GATA-3 and T-bet, which are important in T helper type 1 (Th1) and Th2 cell development, respectively.

Gene expression analysis of peripheral blood mononuclear cells (PBMCs) was performed using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Plasma levels of IFN- $\gamma$  and IL-4 were also determined by ELISA.

T-bet and IFN- $\gamma$  gene expression was significantly lower in patients group compared with healthy controls ( $p < 0.05$ ). The expression of GATA-3 was relatively similar in patients and controls; however, IL-4 mRNAs were significantly increased in the PBMCs from patients as compared with normal controls ( $p < 0.05$ ). In addition, a marked increase in plasma IL-4 levels were observed in patient group compared with controls ( $p < 0.001$ ). To the contrary, IFN- $\gamma$  protein levels were decreased in patients in comparison with controls ( $p < 0.001$ ).

These data suggest additional implications of the role of Th1/Th2 imbalance for the immunopathogenesis of type 1 diabetes.

**Keywords:** Autoimmunity; Cytokine; Type 1 diabetes; T lymphocyte

### **INTRODUCTION**

Type 1 diabetes (T1D), also recognized as Insulin-dependent diabetes mellitus (IDDM) is a chronic immune-mediated disease resulting from selective

destruction of insulin producing beta-cells.<sup>1</sup> It is well known that both cellular and humoral immunities have been implicated in the development of T1D, none the less, accumulated evidence points to an important role for cell-mediated immunity in the immunopathogenesis of beta cell destruction that results in T1D. The significance of T cells in disease development is supported by different observations: 1) the activation of T-lymphocytes against pancreatic  $\beta$ -cells<sup>2</sup>, 2) the

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prominent infiltration of the pancreas by CD4+ and CD8+ T lymphocytes<sup>3,3</sup> diabetes transfer with T cells in non-obese diabetic (NOD) mice as a model for T1D and prevention of diabetes by elimination of CD4+ or CD8+ T cells<sup>4,5</sup>, 4) cytotoxic effects of T cell cytokines on human pancreatic islet cells and protective properties of their inhibitors against islet cells destruction<sup>6,7</sup>, and 5) the efficacy of immunosuppressants for disease control.<sup>8</sup>

Among different subsets of CD4+ T cells, T helper type 1 (Th1) and Th2 cells can participate in promoting or inhibiting of multiple stages of the immune response. There are various pathways that lead to the development of Th1 or Th2 subpopulations. T-bet and GATA-3 are two important transcription factors involved in development of effector Th1 and Th2 cells, respectively. Th1 and Th2 cells also create vastly different profiles of cytokines. For instance, Th1 cells predominantly secrete IL-2 and interferon-gamma (IFN- $\gamma$ ), whereas Th2 cells produce IL-4, IL-5, IL-10, and IL-13.<sup>9</sup> In accordance with existing information, Th1 and Th2 cells contribute to the pathogenesis of different autoimmune diseases, including T1D. However, there is controversy in the literature about T1D being a Th1 or Th2 autoimmune phenomenon, or both.<sup>10-13</sup> Therefore, trying to unravel a specific role for different Th1 and Th2 cells in T1D represent a helpful research aim to further study the immunological background of this important endocrine disorder.

The goal of this research was: 1. To determine T-bet, GATA-3, IFN- $\gamma$ , and IL-4 mRNA expression patterns in peripheral blood mononuclear cells (PBMCs) of patients with T1D and healthy control subjects, 2. Analysis of correlation between T-bet/GATA-3 and IFN- $\gamma$ /IL-4 to understand the potential role of these two transcription factors in signaling for the differentiation of Th1/Th2 lymphocytes, 3. To assess whether there is a difference in cytokine levels (IFN- $\gamma$  and IL-4) between healthy subjects and patients with T1D.

### MATERIALS AND METHODS

#### Subjects

Venous blood (5 ml) were drawn from 21 patients with T1D (9 men, 12 women) in endocrinology clinics and 22 healthy individuals (5 men, 17 women). Blood samples were collected in evacuated glass tubes containing ethylenediaminetetraacetic acid

(EDTA). All patients with T1D received conventional therapy with one or two daily insulin injections. The mean age of the patients in this study was 26.67 $\pm$ 4.78 years (men 25 $\pm$ 4.06; women 27.92 $\pm$ 5.05). Healthy subjects had no history of T1D or other chronic and autoimmune diseases and their mean ages were 29.18 $\pm$ 7.66 years (men, 25.20 $\pm$ 5.63, women, 30.35 $\pm$ 7.91). The protocol was approved by the Research Ethics Board at Tehran University of Medical Sciences (no.IR.TUMS.REC.2854). All patients and control subjects had completed written informed consent before the start of any study-related procedures. PBMCs were isolated by Ficoll density-gradient centrifugation (Pharmacia, Uppsala, Sweden) from the whole blood.

#### RNA Extraction from Peripheral Blood Mononuclear Cells and Reverse Transcription

Total RNA isolation from PBMCs was performed using Ribospin<sup>TM</sup> (GeneALL, Seoul, Korea). Then, the yield and quality of isolated RNA was determined by agarose gel electrophoresis and ethidium bromide staining. Moreover, the ratio of absorbance at 260 nm and 280 nm was used to assess the RNA concentration by Nanodrop spectrophotometer (Thermo Scientific, USA). Only high-quality RNA, with the absorbance ratio between 200 and 400 ng/ $\mu$ l, was used for further examinations. The first strand cDNA was generated from nearly 1000 nanogram (ng) of RNA using the first strand cDNA synthesis kit (Fermentas, Germany) and was kept frozen at -20°C until use.

#### Reverse Transcription-quantitative Polymerase Chain Reaction Analysis

Aliquots of cDNA samples (0.5  $\mu$ g for each) from patients and normal subjects were amplified by SYBR Green I-based reverse transcription-quantitative polymerase chain reaction (RT-qPCR) on a Rotor Gene 6000 (Corbett Life Science, Australia) thermal cycler. Melting curves were created for each RT-qPCR to confirm the specificity of each PCR product.

Primers specific for T-bet, GATA-3, IFN- $\gamma$ , IL-4 and housekeeping gene  $\beta$ -actin were purchased from TAG Copenhagen (Denmark). The exact primers sequences and RT-qPCR protocols utilized in this study was described previously.<sup>14</sup>  $\beta$ -actin gene was used for the normalization of gene expression data. Each reaction was characterized by Ct (cycle threshold) value, at which the fluorescence signal exceeded a

defined background threshold.

The Ct value is correlated to the amount of target mRNA. A greater amount of mRNA target results in a lower number of PCR cycles needed for the reporter fluorescent emission to reach the threshold, and consequently a lower Ct value. The expression level of the target genes were calculated using the  $\Delta$ Ct method. The  $\Delta$ Ct values ( $\Delta$ Ct=Ct value of the target gene – Ct value of the  $\beta$ -actin gene) was statistically analysed in order to determine if any of the differences in gene expression were statistically significant. All statistical analyses were carried out on  $\Delta$ Ct values. Higher  $\Delta$ CT values represent lower mRNA levels. In addition, fold change of expression was calculated by  $2^{-\Delta\Delta Ct}$  method.

### Enzyme-Linked Immunosorbent Assay

Serum IL-4 and IFN- $\gamma$  levels were determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (Bender Med Systems, San Diego, California, USA).

### Statistical Analysis

Data analysis was done with the SPSS, version 11.0 (SPSS, Inc., Chicago, IL, USA). Independent samples t-test was applied to compare two groups of data. Correlations among pairs of variables were assessed by Pearson test. *p*-values less than 0.05 were considered to be significant. Results are reported as means $\pm$ standard deviation (SD).

## RESULTS

### Down-regulated Expressions of T-bet and IFN- $\gamma$ mRNA in Peripheral Blood Mononuclear Cells of Patients with Type 1 Diabetes

The expressions of T-bet and IFN- $\gamma$  in PBMCs of patients were investigated. As shown in Figure 1, compared with healthy subjects, the expression of T-bet mRNA revealed a significant decrease (*p*<0.001) in patients' PBMCs (13.04 $\pm$ 5.35) than in healthy controls (8.46 $\pm$ 1.26). Consistently, the expression of pro-inflammatory cytokines IFN- $\gamma$  (Figure 2), were also 2-fold lower (*p*=0.03) in patients (11.25 $\pm$ 1.91) than in normal controls (10.17 $\pm$ 1.29).

Correlation analyses indicated that T-bet mRNA levels were positively correlated with IFN- $\gamma$  mRNA expression (*r*=0.786; *p*<0.001). IFN- $\gamma$  mRNA expression was also significantly correlated with

plasma IFN- $\gamma$  secretion (*r*=0.459; *p*=0.04).

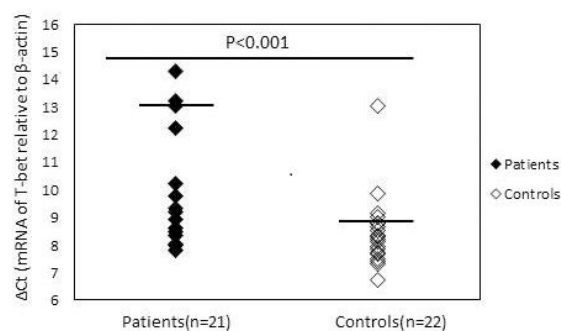
### GATA-3 and IL-4 mRNA Levels Were Higher in Patients with Type 1 Diabetes Compared with Healthy Subjects

RT-qPCR showed no significant difference between patients (12.15 $\pm$ 1.83) and controls (12.34 $\pm$ 2.41) groups for the level of GATA-3 mRNA expression (*p*>0.05). In contrast, the levels of IL-4 mRNA showed a significant 6-fold increase in patients (6.44 $\pm$ 1.27) compared with controls (9.09 $\pm$ 1.30) (*p*<0.001). The mRNA relative expression values for GATA-3 and IL-4 are demonstrated in Figure 3 and Figure 4, respectively.

Correlation analyses revealed no significant correlation between GATA-3 mRNA levels and IL-4 mRNA expression. However, significant positive correlation existed between IL-4 transcript and plasma IL-4 protein levels (*r*=0.703; *p*=0.001).

### Increased Plasma Levels of IL-4 Cytokine in Patients with Type 1 Diabetes

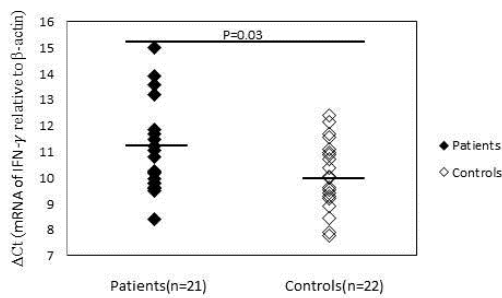
Circulating plasma levels of the cytokines IL-4 and IFN- $\gamma$  were determined in 20 T1D patients and 20 healthy controls. As shown in Figure 5, circulating plasma levels of IL-4 were



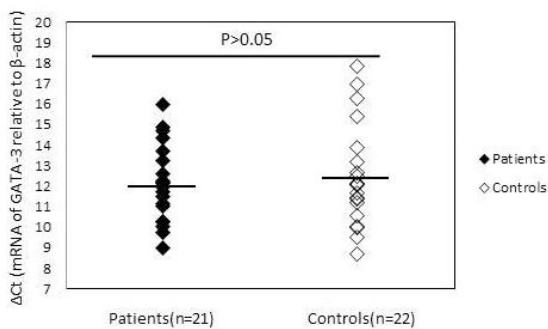
**Figure 1. Detection of T-bet mRNA in peripheral blood mononuclear cells from patients with type 1 diabetes and healthy persons by reverse transcription-quantitative PCR. T-bet mRNA levels were significantly lower in patients than normal persons.**

Please note that a higher  $\Delta$ Ct value corresponds to a comparably lower expression level.

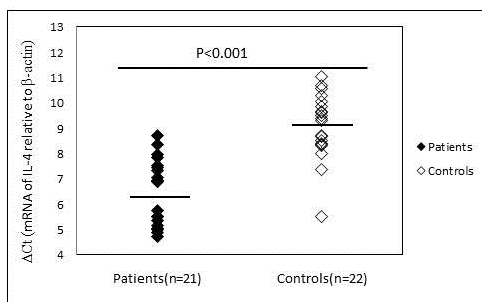
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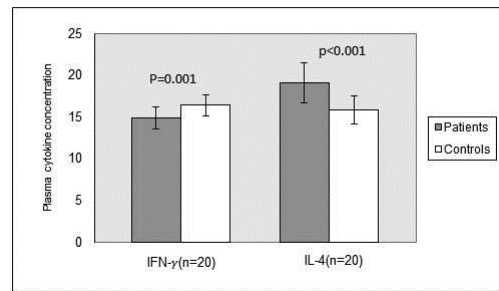
**Figure 2.-** Detection of IFN- $\gamma$  mRNA in peripheral blood mononuclear cells from patients with type 1 diabetes and healthy persons by reverse transcription-quantitative PCR. IFN- $\gamma$  mRNA levels were significantly lower in patients than normal persons. Please note that a higher  $\Delta$ Ct value corresponds to a comparably lower expression level.



**Figure 3.** Detection of GATA-3 mRNA in peripheral blood mononuclear cells from patients with type 1 diabetes and healthy persons by reverse transcription-quantitative PCR. GATA-3 mRNA levels were relatively similar in patients and controls. Please note that a higher  $\Delta$ Ct value corresponds to a comparably lower expression level.



**Figure 4.** Detection of IL-4 mRNA in peripheral blood mononuclear cells from patients with type 1 diabetes and healthy persons by reverse transcription-quantitative PCR. IL-4 mRNA levels were significantly higher in patients than normal persons. Please note that a higher  $\Delta$ Ct value corresponds to a comparably lower expression level.



**Figure 5.** Detection of IFN- $\gamma$  and IL-4 in samples obtained from subjects with type 1 diabetes and healthy subjects, using ELISA. Circulating plasma levels of IL-4 were significantly higher in patients compared with the control group. In contrast, plasma levels of IFN- $\gamma$  were significantly lower in patients than those in the controls.

dramatically higher in patients ( $19.08 \pm 2.42$ ) compared with the control group ( $15.79 \pm 1.71$ ;  $p < 0.001$ ). To the contrary, IFN- $\gamma$  protein levels were decreased in patients ( $14.85 \pm 1.31$ ) in comparison with controls ( $16.36 \pm 1.24$ ;  $p = 0.001$ ). Moreover, Pearson correlation analysis revealed that IL-4 mRNA levels of T1D patients were positively correlated with the plasma IL-4 levels ( $r = 0.703$ ,  $p = 0.001$ ). Significant correlation were found between plasma IFN- $\gamma$  concentration and IFN- $\gamma$  mRNA expression ( $r = 0.459$ ;  $p = 0.04$ ). However, no significant correlation was observed between T-bet mRNA expression and plasma IFN- $\gamma$  concentration ( $p > 0.05$ ).

## DISCUSSION

T1D is a wide spread chronic endocrine disorder that results in the destruction of the insulin-producing beta cells in the pancreatic islets of Langerhans and has been on the rise since the 20<sup>th</sup> century.<sup>15</sup> The etiology of the autoimmune attack in this life-debilitating human disorder remains unknown but T cells appear to play a pivotal role in the pathophysiology of T1D. The precise identity of T cell subset(s) involved in the pathogenesis of disease remains unknown because of their heterogeneity and plasticity (the ability of a cell to change its phenotype). Therefore, one important question that needs to be addressed is identification of the specific T cell subtype(s) mediating an autoimmune response in patients.

Several studies suggest that disruption of T cell tolerance and dysregulation between Th1 and Th2 T cell subpopulations play a major role in the pathogenesis and development of T1D.<sup>10-13</sup> It is well known that T-bet and GATA-3 are two critical transcription factors during the early polarization of Th1 and Th2 cells and their altered expression levels appear to influence Th1/Th2 balance. Our results indicated a considerable decrease in T-bet mRNA level in PBMCs of patients compared with normal controls. On the contrary, the levels of GATA-3 specific mRNA did not differ between samples taken from diabetic patients and healthy controls.

To the best of our knowledge, this is the first report on the analysis of T-bet and GATA-3 in PBMCs of adult patients suffering from T1D. These data suggest poor Th1 immune activation in peripheral blood of the adult patients with clinical diabetes and these findings are consistent with earlier studies showing decreased expression of T-bet transcripts in peripheral blood of children with T1D.<sup>12</sup> Therefore, gene expression changes of T-bet and GATA-3 mRNA may be implicated in the pathogenesis of T1D. In the second part of this study, the expression levels of IFN- $\gamma$  and IL-4 were assessed. Moreover, the plasma levels of these cytokines were also measured by ELISA. There were two important points for cytokine gene expression analysis. Firstly, Th2 and Th1 cells are the primary cellular source of IL-4 and IFN- $\gamma$  production, respectively and altered production of these cytokines plays important roles in pathogenic host immune responses. Secondly, considerable controversy exists in the literature over the role that IL-4 and IFN- $\gamma$  play in T1D. Our results indicated a major decrease of IFN- $\gamma$  mRNA level in patients compared with normal controls. Moreover, the level of T-bet was significantly correlated with IFN- $\gamma$  mRNA expression. IFN- $\gamma$  mRNA expression was also significantly correlated with plasma IFN- $\gamma$  secretion. These data are in good agreement with earlier studies indicating reduced expression of IFN- $\gamma$  transcripts in peripheral blood of diabetic patients.<sup>16</sup> Nonetheless, these results are also in contrast to some other findings stating a major pathogenic role of Th1 cells and related cytokines in T1D.<sup>17</sup> In our study, the IL-4 gene was another candidate for measuring mRNA expression. In contrast to IFN- $\gamma$ , IL-4 mRNA levels and plasma IL-4 secretion were significantly higher in patients than in controls. Moreover, there was also good correlation between

IL-4 transcript and plasma IL-4 protein levels. These findings are consistent with previous researches, suggesting a protective role of IL-4 in T1D.<sup>18</sup> For instance, Cetkovic-Cvrlje et al. reported decreased diabetes incidence in NOD mice, by injecting candidate  $\beta$ -cell autoantigen [whole islet cells, glutamic acid decarboxylase 65(GAD65), insulin B chain] into the thymus. They indicated that this protection was associated with enhanced IL-4 and IL-10 expression by Th2 cells.<sup>19</sup> In addition, correlations have been observed between benign insulinitis and expression of type 2 cytokines including IL-4.<sup>18</sup> The term benign insulinitis has been utilized to explain the accumulation of mononuclear leukocytes around and within pancreatic islets, without progressive destruction of insulin-producing beta cells.<sup>20</sup> Moreover, IL-4 expression in beta cells of transgenic mice leads to strong protection against insulinitis and diabetes.<sup>21</sup> Additionally, systemic administration of IL-4 have shown to prevent T1D progression in NOD mice, because deficiency of endogenous IL-4 production has been reported in these mice.<sup>22</sup>

The mechanism of protection mediated by IL-4 still remains to be unknown and this cytokine probably exerts its influence through various pathways. One possible mechanism involves the downregulation of diabetogenic T-cells through intraislet production of IL-4. It seems that, IL-4 exposure could eliminate enhanced autoreactive responses and induction of islet antigen-specific memory effectors via reduced expression of Fc- $\gamma$  receptor I (Fc $\gamma$ RI) on macrophages.<sup>23</sup> Moreover, IL-4 is an anti-inflammatory cytokine which crossregulates the production of IFN- $\gamma$ .<sup>24</sup> The results of present study, do not confirm the outcomes of previous researches pointing to the pathogenic role of Th1 cells in T1D<sup>18</sup>. These disparities may be dependent upon patient related factors specially their ages, various steps of the autoimmune inflammatory response and treatment that may affect some aspects of the immune system which may cause an altered Th1/Th2-balance and the cytokines they release. Several studies have shown the impact of different therapeutic approaches on the immune system of diabetic patients. For instance, elevated numbers of IL-4 and IL-10-producing cells were shown in islets of NOD mice protected against diabetes progression by different treatments, such as oral delivery of insulin<sup>25</sup>, footpad inoculation with complete Freund's adjuvant<sup>26</sup> and IL-10/Fc fusion protein injection through the

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intraperitoneal route.<sup>27</sup>

As mentioned above, patients enrolled in this experiment were under insulin therapy. Insulin injection is the predominant treatment for T1D and its protection may be achieved by different mechanisms. Insulin is effective to relieve hyperglycemia and may influence immune system activity. It seems to be able to modulate autoimmunity reflected by elevated levels of circulating insulin antibodies<sup>28</sup>, giving beta cells a rest or down-regulate the expression of autoantigens in beta-cells.<sup>29,30</sup> It has been shown that exogenous insulin administration can reduce T-cell reactivity to insulin and insulin peptides. Therefore, insulin injection may lead to a delay or even prevention of autoimmune diabetes.<sup>31</sup> These findings suggest that better blood sugar control can improve the capacity of activation and maintenance of the immune response.

Disease duration, patients' age and the influence of metabolic control in diabetic patients are other important factors that must be considered. These concepts were discussed by different researchers.<sup>32,33</sup> For instance, decreased amounts of IL-4 and TGF- $\beta$  were found in the children with newly diagnosed diabetes and in prediabetic children. Altered expression of immune-related genes was also reported in patients with a longstanding history of T1D<sup>34</sup> Overall, these studies together with our present results provide evidences for the Th1/Th2 imbalance in patients with T1D receiving insulin treatment.

Nonetheless, we believe that splitting complex diseases such as T1D, in terms of Th1 and Th2 patterns, is an oversimplification because the development of a given Th cell set and, in turn, the outcome of the diabetogenic response certainly involves the cooperation between different immune cell types and factors. For example, CD4+ Th1 autoreactive clones have been isolated from the spleen of unprimed NOD mice with regulatory activity. These cells can suppress the adoptive transfer of diabetes.<sup>35</sup> Moreover, T cells expressing a diabetogenic T cell receptor (TCR) when cultured under appropriate conditions that promote Th2 development were unable to mediate protection in NOD recipients.<sup>36</sup> In addition, the role of other cells such as CD8+ cells that exhibit Th1- and Th2-like phenotypes, invariant natural killer T (iNKT) cells and cytokines secreted by non-T cells must undoubtedly be considered.<sup>37,38</sup>

Attention to studies addressing the role of different immune cells and even non-immune cells in IDDM

seems to be also a response to some of the findings of the current study. For instance, in the present study there was no correlation between GATA-3 and IL-4 mRNA expression. According to our opinion this discordance may stem from the existence of different types of mononuclear cells that produce IL-4 including B cells, NK cells and regulatory T cells. For instance, Ghazarian, et al. indicated that IL-4 production by NKT cells are critical for the dampening of pathogenic anti-islet T cell responses.<sup>39</sup> The therapeutic effect of zymosan in experimental model of IDDM was also attributed to increased frequency of IL-10-, IL-17-, IL-4-, and Foxp3-positive T cells.<sup>40</sup>

Our results describe a Th2-skewed immune response in adult patients with T1D receiving insulin therapy. This implies the activation of various T-cell subpopulations in patients with respect to insulin treatment. Whether this therapeutic approach in diabetic patients can stimulate the development of non-destructive, or even protective T-cell subpopulations must be analyzed in further investigations. In addition, future studies are required to elucidate changes in which subgroups of heterogenous populations of T cells are associated with diabetes duration and how the therapy influences their frequency and function.

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### REFERENCES

1. Taborsky GJ Jr, Ahrén B, Havel PJ. Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired alpha-cell responses in type 1 diabetes. *Diabetes* 1998; 47(7):995-1005.
2. Boitard C. Pancreatic islet autoimmunity. *Presse Med* 2012; 41:e636-50.
3. Boitard C. T-lymphocyte recognition of beta cells in type 1 diabetes: clinical perspectives. *Diabetes Metab* 2013; 39(6):459-66.
4. Berry G, Waldner H. Accelerated type 1 diabetes induction in mice by adoptive transfer of diabetogenic CD4+ T cells. *J Vis Exp* 2013; 75:e50389.

5. van Belle TL, Coppieters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev* 2011; 91(1):79-118.
6. Soldevila G, Buscema M, Doshi M, James RF, Bottazzo GF, Pujol-Borrell R. Cytotoxic effect of IFN-gamma plus TNF-alpha on human islet cells. *J Autoimmun* 1991; 4(2):291-306.
7. Rabinovitch A, Baquerizo H, Sumoski W. Cytotoxic effects of cytokines on islet beta-cells: evidence for involvement of eicosanoids. *Endocrinology* 1990; 126(1):67-71.
8. Bach JF, Chatenoud L. A historical view from thirty eventful years of immunotherapy in autoimmune diabetes. *Semin Immunol* 2011; 23(3):174-81.
9. Zhang Y, Zhang Y, Gu W, Sun B. TH1/TH2 cell differentiation and molecular signals. *Adv Exp Med Biol* 2014; 841:15-44.
10. Hultgren B, Huang X, Dybdal N, Stewart TA. Genetic absence of gamma-interferon delays but does not prevent diabetes in NOD mice. *Diabetes* 1996; 45(6):812-7.
11. Karlsson Faresjö MG, Ludvigsson J. Diminished Th1-like response to autoantigens in children with a high risk of developing type 1 diabetes. *Scand J Immunol* 2005; 61(2):173-9.
12. Walldén J, Honkanen J, Ilonen J, Ludvigsson J, Vaarala O. No evidence for activation of T(H)1 or T(H)17 pathways in unstimulated peripheral blood mononuclear cells from children with  $\beta$ -cell autoimmunity or T1D. *J Inflamm Res* 2008; 1:11-7.
13. Csorba TR, Lyon AW, Hollenberg MD. Autoimmunity and the pathogenesis of type 1 diabetes. *Crit Rev Clin Lab Sci* 2010; 47(2):51-71.
14. Vanaki E, Ataei M, Sanati MH, Mansouri P, Mahmoudi M, Zarei F, et al. Expression patterns of Th1/Th2 transcription factors in patients with guttate psoriasis. *Acta Microbiol Immunol Hung* 2013; 60(2):163-74.
15. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009. *JAMA* 2014; 311(17):1778-86.
16. Halminen M, Simell O, Knip M, Ilonen J. Cytokine expression in unstimulated PBMC of children with type 1 diabetes and subjects positive for diabetes-associated autoantibodies. *Scand J Immunol* 2001; 53(5):510-3.
17. Marwaha AK, Tan S, Dutz JP. Targeting the IL-17/IFN- $\gamma$  axis as a potential new clinical therapy for type 1 diabetes. *Clin Immunol* 2014; 154(1):84-9.
18. Rabinovitch A. An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. *Diabetes Metab Rev* 1998; 14(2):129-51.
19. Cetkovic-Cvrlje M, Gerling IC, Muir A, Atkinson MA, Elliott JF, Leiter EH. Retardation or acceleration of diabetes in NOD/Lt mice mediated by intrathymic administration of candidate beta-cell antigens. *Diabetes* 1997; 46(12):1975-82.
20. Kolb H. Benign versus destructive insulinitis. *Diabetes Metab Rev* 1997; 13(3):139-46.
21. Mueller R, Krahl T, Sarvetnick N. Pancreatic expression of interleukin-4 abrogates insulinitis and autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med* 1996; 184(3):1093-9.
22. Rapoport MJ, Jaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, et al. Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J Exp Med* 1993; 178(1):87-99.
23. Mueller R, Bradley LM, Krahl T, Sarvetnick N. Mechanism underlying counterregulation of autoimmune diabetes by IL-4. *Immunity* 1997; 7(3):411-8.
24. Russell MA, Morgan NG. The impact of anti-inflammatory cytokines on the pancreatic  $\beta$ -cell. *Islets* 2014; 6(3):e950547.
25. Hancock WW, Polanski M, Zhang J, Blogg N, Weiner HL. Suppression of insulinitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta, and prostaglandin-E. *Am J Pathol* 1995; 147(5):1193-9.
26. Suarez-Pinzon W, Rajotte RV, Mosmann TR, Rabinovitch A. Both CD4+ and CD8+ T-cells in syngeneic islet grafts in NOD mice produce interferon-gamma during beta-cell destruction. *Diabetes* 1996; 45(10):1350-7.
27. Zheng XX, Steele AW, Hancock WW, Stevens AC, Nickerson PW, Roy-Chaudhury P, et al. A noncytolytic IL-10/Fc fusion protein prevents diabetes, blocks autoimmunity, and promotes suppressor phenomena in NOD mice. *J Immunol* 1997; 158(9):4507-13.
28. Deckert T. The immunogenicity of new insulins. *Diabetes* 1985; (34 Suppl 2):94-6.
29. Schloot N, Eisenbarth GS. Isohormonal therapy of endocrine autoimmunity. *Immunol Today* 1995; 16(6):289-94.
30. Song YH, Li Y, Maclaren NK. The nature of autoantigens targeted in autoimmune endocrine diseases. *Immunol Today* 1996; 17(5):232-8.
31. Schloot NC, Roep BO, Wegmann D, Yu L, Chase HP, Wang T, et al. Altered immune response to insulin in newly diagnosed compared to insulin-treated diabetic patients and healthy control subjects. *Diabetologia* 1997;

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- 40(5):564-72.
32. Chatzigeorgiou A, Harokopos V, Mylona-Karagianni C, Tsouvalas E, Aidinis V, Kamper EF. The pattern of inflammatory/anti-inflammatory cytokines and chemokines in type 1 diabetic patients over time. *Ann Med* 2010; 42(6):426-38.
  33. Rydén A, Ludvigsson J, Fredrikson M, Faresjö M. General immune dampening is associated with disturbed metabolism at diagnosis of type 1 diabetes. *Pediatr Res* 2014; 75(1-1):45-50.
  34. Planas R, Carrillo J, Sanchez A, de Villa MC, Nuñez F, Verdaguer J, et al. Gene expression profiles for the human pancreas and purified islets in type 1 diabetes: new findings at clinical onset and in long-standing diabetes. *Clin Exp Immunol* 2010; 159(1):23-44.
  35. Akhtar I, Gold JP, Pan LY, Ferrara JL, Yang XD, Kim JI, et al. CD4<sup>+</sup> beta islet cell-reactive T cell clones that suppress autoimmune diabetes in nonobese diabetic mice. *J Exp Med* 1995; 182(1):87-97.
  36. Katz JD, Benoist C, Mathis D. T helper cell subsets in insulin-dependent diabetes. *Science* 1995; 268(5214):1185-8.
  37. Kis J, Engelmann P, Farkas K, Engelmann P, Farkas K, Jalahej H, et al. Reduced CD4<sup>+</sup> subset and Th1 bias of the human iNKT cells in Type 1 diabetes mellitus. *J Leukoc Biol* 2007; 81(3):654-62.
  38. Tisch R, McDevitt H. Insulin-dependent diabetes mellitus. *Cell* 1996; 85(3):291-7.
  39. Ghazarian L, Simoni Y, Pingris K, Beaudoin L, Lehuen A. Regulatory role of NKT cells in the prevention of type 1 diabetes. *Med Sci (Paris)* 2013; 29(8-9):722-8.
  40. Karumuthil-Meethil S, Sofi MH, Gudi R, Johnson BM, Perez N, Vasu C. TLR2- and Dectin 1-associated innate immune response modulates T-cell response to pancreatic  $\beta$ -cell antigen and prevents type 1 diabetes. *Diabetes* 2015; 64(4):1341-57.