IL-4 AND INTERFERON GAMMA IN RECENTLY DIAGNOSED TYPE I DIABETES, A CASE-CONTROL STUDY

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Abstract

INTRODUCTION: The aim of this study was to measure serum levels of interferon-gamma (IFN-γ) and interleukin-4 (IL-4), the two major cytokines secreted by Th-1 and Th-2 cells, in islet cell autoantibody (ICA)-positive, newly-diagnosed type I diabetic patients.

METHODS: The study was conducted on 30 newly diagnosed, ICA-positive type I diabetics and 30 age- and sex-matched healthy controls. Cytokine levels in serum were quantified by indirect sandwich ELISA in pg/ml.

RESULTS: We observed no significant difference in concentration of IL-4 in ICA-positive diabetics (median=126.535) compared with healthy controls (median=136.440) ($P>0.05$). IFN-γ levels were significantly higher in patients (median=11.305) compared with healthy controls (median=8.200) ($P<0.05$).

CONCLUSIONS: Increased levels of IFN-γ in patients may be suggestive of its destructive role in the pathophysiology of type I autoimmune diabetes.

Keywords: Type I diabetes mellitus, interleukin-4 (IL-4), interferon-gamma (IFN-γ), islet cell autoantibody (ICA), T-helper 1 (Th-1) response, T-helper 2 (Th-2

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Introduction

Type I autoimmune diabetes accounts for 5-10% of all diabetes cases. The autoimmune response that leads to destruction of pancreatic β-cells in type I autoimmune diabetes mellitus develops from a combination of genetic, environmental and immunologic factors.[1-14]

Various antibodies, such as islet cell autoantibodies (ICA), insulin autoantibodies (IAA), and anti-glutamic acid decarboxylase antibodies (anti-GAD-Ab) are implicated as immunologic markers in type I autoimmune diabetes patients.

Current evidence favors the concept that β-cells are destroyed by an autoimmune response directed against certain β-cell constituents (autoantigens).[15]

T-lymphocytes specific for pancreatic β-cell molecules (i.e. autoantigens) normally exist, but are thought to be restrained by immunoregulatory mechanisms (the self-tolerant state).

Current research has shown that type I autoimmune diabetes develops when one or another immunoregulatory mechanism fails, allowing autoreactive T-cells directed against β-cells to become activated and to expand clonally, starting a cascade of immune/inflammatory processes in the islets (insulitis), culminating in β-cell destruction.

The cytokine profile of T-helper cells is crucial to the development of an effective immune response.

Th-1 cells secrete IL-2, IFN-γ and tumor necrosis factor beta (TNF-β) which may cause diabetes in different ways. These mediators may stimulate immune inflammatory responses such as the activation of cytotoxic T-cells. This, in turn, may lead to destruction of pancreatic β-cells and activation of macrophages leading to the secretion of pro-inflammatory cytokines such as IL-1, TNF-α, IFN-γ and free oxygen or nitrogen radicals which are cytotoxic to β-cells.[16]

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IFN-γ may also sensitize β-cells to the cytotoxicity of T-cells by increasing the expression of the major histocompatibility complex (MHC), class I.
On the other hand, Th-2 cells secrete cytokines such as IL-4 and IL-10 which may be protective for β-cells.[17-20]
IL-4 is the primary cytokine of the Th-2 pathway.[21-23] Along with its ability to drive T-helper cells to a Th-2 phenotype, IL-4 also possesses strong down-regulatory properties with respect to Th-1 cells,[24,25] not excluding autoreactive Th-1 cells.[26,27]
The local expression of IL-4 in the pancreatic islets of NOD mice (ins-IL-4 mice) restricted the activation of autoreactive T-cells and promoted complete protection against spontaneous diabetes.[28]
The expression of IL-4 in the target organ significantly reduced the diabetogenic potential of islet-specific T-cells.
These findings have led to the development of the hypothesis that prevention of type I diabetes can be achieved by inhibition of Th-1 reactions and stimulation of Th-2 reactions and this is the current model upon which investigations and immunotherapeutic interventions are being designed.
However, most studies have been carried out in animal models for human IDDM-NOD mice and BB rats. The evidence that this Th1/Th2 paradigm applies to the pathogenesis of human IDDM is limited. The possible roles of cytokines in the pathogenesis of the human disease are less well characterized. Histological studies of the pancreas of humans with IDDM have been limited by necessity, to patients in whom clinical diabetes has already developed, and in these patients the insulitis lesion is likely near, or at an end stage. In this situation, IFN-α and IFN-γ, but not other cytokines, have been detected in human islets.[29-32]
IFN-γ produced by T-cells that infiltrate human islets[32] and possibly macrophage-derived IL-1 and TNF-α, may be directly cytotoxic to human islet β-cells *in viv*o, as demonstrated for these cytokines *in vitr*o.[33,34] In human, currently the only defensible claims are that the Th1 cells produce far more IFN-γ and IL-12 than do Th2 cells, while the Th2 cells produce far more IL-4 (and perhaps IL-5) than do the Th1 cells.
In this study, we used ELISA to measure IL-4 and IFN-γ serum levels in 30 newly diagnosed type I autoimmune diabetics and compared them with the same levels in 30 healthy controls. Considering the genetic and environmental factors which may be different in our population from the others, this study may provide new information about our population.

Materials and methods

This cross-sectional study was conducted in the Bu-Ali Research Institute and the Khorasan Diabetes Research Center (Mashhad, Iran) from March 2003 to September 2004. Serum samples were procured from 30 ICA-positive Iranian patients among 43 newly-diagnosed type I diabetics (less than one year from the time of diagnosis) in the Khorasan Diabetes Research Center. Qualitative ELISA test for detection of circulating autoantibodies against islet cell antigens (Isletest-ICA BIOMERICA) was used.
Our criteria for diagnosis of type I diabetes followed the 1997 American Diabetes Association (ADA) recommendations.
Patients with type I diabetes were defined on the basis of florid presentation of classical symptoms (polyuria, polydipsia and weight loss) or diabetic ketoacidosis, both requiring insulin treatment. The mean age of the study participants was 13.3 years.
Eleven patients were female and 19 were male. These patients were all from the province of Khorasan and from the same cultural, ethnic, and economic backgrounds.
The control group included 10 healthy female and 20 healthy male subjects, selected from among the students of high schools and primary schools in Ghasem-Abad, which is a suburb of Mashhad. They had a mean age of 13.8 years and shared the same cultural, ethnic, and economic backgrounds as the patients. The study subjects had no family history of diabetes. Inflammatory diseases were ruled out in this group based on medical history, physical examination and negative C-reactive protein (CRP) (a qualitative test). Serum samples were extracted and kept at -70°C until assayed.
The cytokines IFN-γ and IL-4 were determined using high-sensitivity ELISA (Bender MedSystems, Austria). The detection limits for IL-4 and IFN-γ were 2.0 pg/ml and 1.5 pg/ml, respectively. The overall inter-assay coefficients of variation for IL-4 and IFN-γ were 4.8% and 4.5%, respectively. All of the participants gave their informed consent before being included in the study.
IFN-γ serum levels (Figure 3) had bell-shaped distribution in both cases and controls. The IL-4 serum levels (Figure 2) in controls also had a bell-shape distribution, but a slight deviation from the bell shape was observed in cases; although this deviation

did not have a statistically significant influence on the results when analyzed by t-test for independent samples, we used the Wilcoxon rank sum test to analyze the data more accurately. P values less than 0.05 were considered as statistically significant.

Results

Among 43 newly diagnosed type I diabetics, 30 were ICA-positive (69.7%). The mean age in this group was 13.3 years, with a range from 4 to 22 years.

Eleven cases were female and 19 were male (Table 1). All patients were selected at the Khorasan Diabetes Center and had the same ethnic, cultural, and economic backgrounds.

Our control group included 30 healthy CRP-negative individuals with a mean age of 13.8 years. Ten subjects were female and 20 were male (Table 1); they were selected from among high school students in Ghasem-Abad which is a suburb of Mashhad. Inflammatory conditions and a family history of diabetes were ruled out in this group by taking history and performing physical examination. Blood samples were obtained and qualitative serum CRP detection test was performed; subjects with negative CRP were selected. The median IL-4 serum level in cases (median: 126.535) was not significantly different from that in controls (median: 136.440) ($P<0.05$) (Figure1). The median IFN-γ serum level in cases (median: 11.305) was significantly higher than that in controls (median: 8.200) ($P<0.05$) (Figure 1). This may indicate the predominance of the Th-1 response in patients compared to healthy individuals.

Discussion

We determined the serum levels of IL-4 and IFN-γ in 30 recently diagnosed ICA-positive type I diabetics.

The results were compared with corresponding levels in 30 age- and sex-matched healthy controls. Any possible condition leading to an active immunologic response (other than diabetes in the patients) was ruled out by taking history and performing a complete physical examination in both cases and controls. We also performed qualitative CRP test in the controls and selected those with negative CRP.

The results showed no significant difference in serum IL-4 levels between the cases (median: 126.535) and the age- and sex-matched healthy CRP-negative controls (median: 136.440) ($P<0.05$) (Figure1).

TABLE 1. Characteristics of the two study groups.

Group	Number	Gender	Mean age at the time of study	Age range at the time of study	Mean time between diagnosis and the study	Median IL-4 serum levels	Median IFN-gamma serum levels
Patients	30 ICA-Positive diabetics out of total 43 diabetics (%69.7)	F=11 M=19	13.3	4-24 years	9 months (1-12 months)	126.535	11.305
Controls	30 Healthy, CRP-Negative controls	F=10 M=20	13.8	7-18 years	-	136.440	8.200

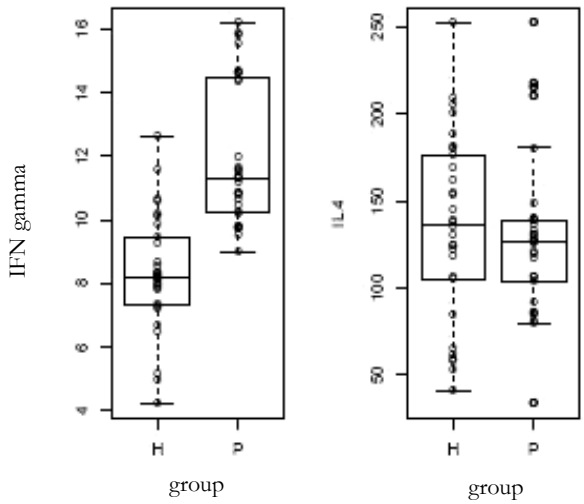

FIGURE 1. IL-4 and IFN-γ serum levels (pg/ml), Wilcoxon rank sum test.

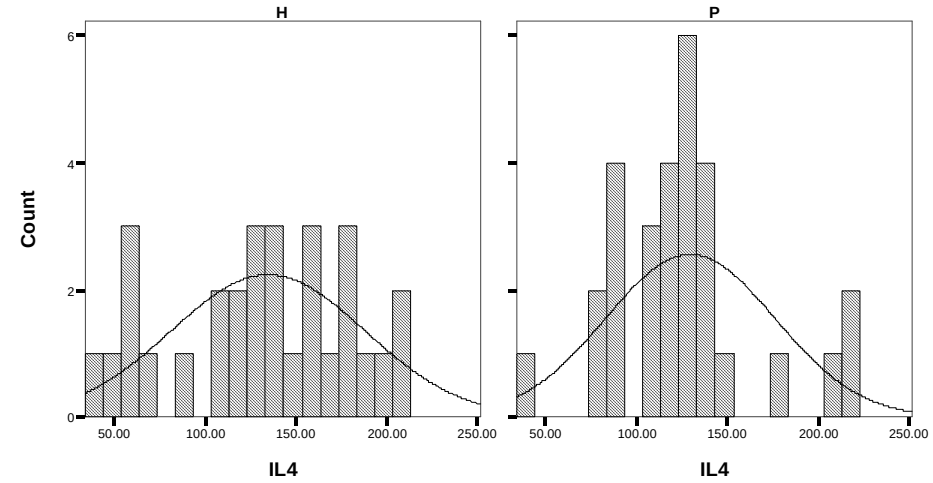

FIGURE 2. Distribution of IL-4 serum levels in cases and controls.

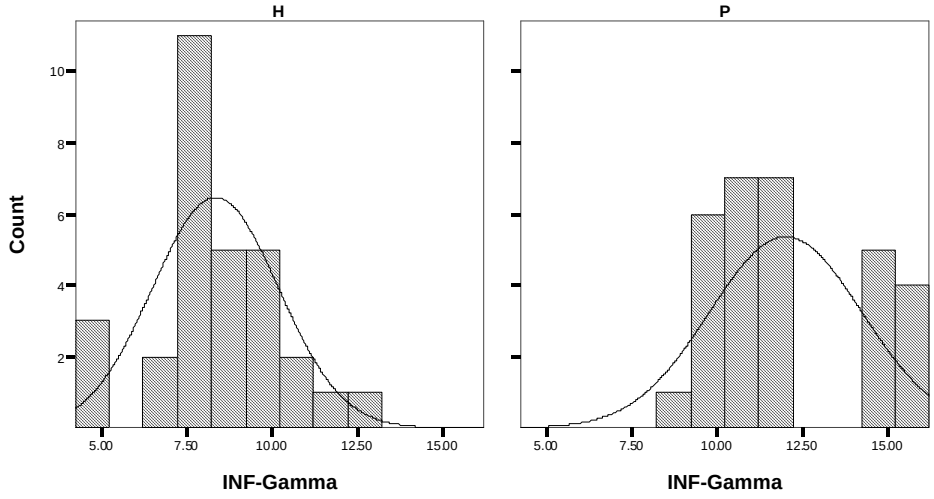

FIGURE 3. Distribution of IFN-γ serum levels in cases and controls.

However, the serum levels of IFN-γ in the cases (median: 11.305) were significantly higher than in controls (median: 8.200) (P<0.05) (Figure 1). This may suggest a destructive role for IFN-γ in the pathophysiology of type I diabetes. It is now well documented that certain cytokines are cytotoxic to pancreatic islets *in vitro*.[33,34] IL-1, IFN-α, TNF-β, and IFN-γ (in piconanomolar concentrations) are *cytostatic* to β-cells; i.e. the individual cytokines inhibit insulin synthesis and secretion, but these functions may recover after the cytokine is removed. In addition, the cytokines can be *cytocidal*; i.e. IL-1, IFN-α, TNF-β and IFN-γ, usually destroy β-cells in both rodent and human islets when added in combination. Because the cytodestructive effects of cytokines on islet β-cells *in vitro* are not selective to β-cells (e.g. β-cells in the islets are also damaged),[34] cytokines may not qualify as mediators of β-cell destruction in IDDM, which is β-cell specific.

Whereas these cytokines are produced by islet-infiltrating macrophages and T-cells in the insulitis lesion of IDDM, it has not been proven that cytokines are directly cytotoxic to β-cell *in viv*o. IFN-γ has been detected by immunohistochemistry in lymphocytes infiltrating islets of human subjects with recent-onset IDDM.[32]

In another study it has been reported that cells in whole blood from patients with IDDM produced significantly higher amounts of Th1 cytokines (IFN-γ and IFN-A) than cells from normal control subjects, while production of Th2 cytokines (IL-4 and IL-10) was similar in diabetic and control subjects. Also, the ratio of IFN-γ and IFN-A to IL-4 or IL-10 was significantly biased toward Th1 reactivity in patients

with IDDM.[37] It is unclear, however, whether changes in production of cytokines by cells from patients with IDDM preceded or resulted from diabetes in these studies.[35-37]

The therapeutic implication of current information on the roles of cytokines in IDDM pathogenesis is that the immune response in IDDM may be diverted from autoimmunity to self-tolerance by intervening in the cytokine network. These may include cytokines, antibodies to cytokines and cytokine receptors, soluble cytokine receptors and receptor antagonists, and receptor-targeted cytotoxic drugs in attempts to block the production and/or action of proinflammatory cytokines (IL-1, IFN-γ and IFN-α) and type I cytokines (IFN-γ, TNF-β, IL-2 and IL-12) that lead to β-cell destruction, and increase the production and/or action of regulatory cytokines (IL-4, IL-10 and TGF-β) that suppress cytotoxic T-cells, macrophages and cytokines. However, information is scarce regarding cytokines expressed in pancreatic islets of human subjects with IDDM.

Systemic (parenteral) administrations of cytokines in diabetes-prone NOD mice and BB rats have, for some cytokines, reproduced the effects of intra-islet cytokine action, whereas for other cytokines, systemic administrations have produced effects opposite to those seen with cytokine action localized to the islet.

For example, IL-1, TNF-α and TGF-β are cytotoxic to islet β-cells *in vitro*,[33,34] whereas systemic administrations of IL-1, TNF-α and TGF-β decrease the incidence of diabetes in NOD mice and/or BB rats.[38-44]

Paradoxically, IFN-γ decreased insulitis in NOD mice when administered together with TNF-α.[45] Furthermore, systemically administered IFN-γ has recently been reported to significantly decrease the incidence of IDDM in BB rats;[46] however, the mechanism(s) underlying the antidiabetogenic effect of mouse IL-4 and IL-10 plasmids prevents the development of autoimmune diabetes in non-obese diabetic (NOD) mice.[47] It has also been reported that human IL-10 is effective in preventing diabetes in NOD mice.[48]

In another study, an anti-IFN-γ monoclonal antibody decreased the incidence of cyclophosphamide-induced diabetes in NOD mice,[49,50] as well as the adoptive transfer of diabetes in adult irradiated, and newborn NOD recipients of spleen cells from diabetic NOD mice.[50] In addition, an anti-IFN-γ polyclonal antibody delayed the onset and decreased the incidence of diabetes in BB rats, even when antibody administration was started at the end of the pre-diabetic period.[51] A limiting factor in the use of antibodies (monoclonal or polyclonal) is the induction of a humoral immune response consisting of antixenotypic and antiidiotypic antibodies capable of reducing the bioactivity of the antibody.

Our findings of significantly increased IFN-γ levels in type I diabetics compared with a healthy control group in our population are in accordance with previous findings about the destructive role of IFN-γ in the process of β-cell destruction,[17-20] and as a consequence, the progression of disease.

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