

IL-10 and IL-13 Production by Peripheral Blood Mononuclear Cells in Patients with Diabetes Mellitus

Ali İNAL¹
Uğur MUŞABAK¹
Göksal KESKİN²
Levent ÖZSARI¹
Ali ŞENGÜL¹
Mustafa KUTLU³
Çağatay ÖKTENLİ¹
Zeki YEŞİLOVA¹

Aim: It is generally accepted that proinflammatory cytokines secreted by mononuclear cells are responsible for pancreatic beta (B) cell destruction in animal models of autoimmune type-1 diabetes mellitus (DM). Several studies have shown that markers of inflammation and pro-inflammatory cytokines such as interleukin (IL)-10 and IL-13 associate with the metabolic syndrome, dyslipidemia, type-2 DM and type-1 DM. The aim of this study was to examine the in vitro production of IL-10 and IL-13 in cultures of peripheral lymphocytes obtained from patients with type-2 DM, type-1 DM and healthy controls.

Materials and Methods: Twenty patients (10 females, 10 males) with type-2 DM (median disease duration: 10 years), 10 patients (5 females, 5 males) with type-1 DM (median disease duration: 11 years) and the healthy control group were enrolled in the study. IL-10 and IL-13 in culture supernatants were measured using the enzyme immunoassay method. Statistical analyses were performed using Mann-Whitney U test, Wilcoxon signed rank tests, and Spearman's rank correlation.

Results: IL-10 and IL-13 levels were similar in culture supernatants incubated with 10 µg/ml phytohemagglutinin (PHA) in all patients and healthy controls ($P > 0.05$). In type-2 DM, IL-10 levels were significantly lower than in the control group in culture supernatants incubated with 20 µg/ml PHA ($P = 0.001$ for poor metabolic control and $P = 0.048$ for good metabolic control, respectively).

Conclusions: According to this study, the alterations in IL-13 did not play an important role in the pathogenesis of type-2 DM and type-1 DM. Furthermore, the deficiency in IL-10 production from peripheral blood mononuclear cells observed with high dosages of PHA suggests that IL-10 may play an important role in the pathogenesis of type-2 DM.

Key Words: IL-10, IL-13, diabetes mellitus

¹ Department of Immunology, Gülhane Military Medical Academy, Ankara - TURKEY

² Department of Immunology, Dışkapı Yıldırım Beyazıt Research and Training Hospital, Ankara - TURKEY

³ Department of Endocrinology, Gülhane Military Medical Academy, Ankara - TURKEY

Diyabetli Hastalarda Periferik Kan Mononükleer Hücrelerinin IL-10 ve IL-13 Üretimi

Amaç: Genel olarak otoimmün tip-1 diabetes mellitus (DM)'lü hayvan modellerinde pankreatik (beta) B hücre destrüksiyonundan mononükleer hücrelerden salgılanan proinflatuvar sitokinlerin sorumlu olduğu kabul edilmektedir. Bazı çalışmalarda metablik sendrom, dislipidemi, tip-2 DM ve tip-1 DM ile inflamasyon göstergeleri ve proinflatuvar sitokinler olan interlökin- (IL)-10 ve IL-13'ün beraberliği gösterilmiştir. Bu çalışmanın amacı, tip-2 DM ve tip-1 DM'li hastalarla sağlıklı kontrollerin periferik kan lenfosit kültürlerinde in vitro IL-10 ve IL-13 üretimini çalışmaktır.

Yöntem ve Gereç: Bu çalışmaya tip-2 diyabetli 20 hasta (10 bayan, 10 erkek; median hastalık süresi 10 yıl), tip-1 diyabetli 10 hasta (5 bayan, 5 erkek; median hastalık süresi: 11 yıl) ve sağlıklı kontrol grubu dahil edildi. Kültür süpernatanlarında IL-10 ve IL-13 düzeyleri enzyim immunoassay metodu ile ölçüldü. İstatistiksel analizler için Mann-Whitney U testi, Wilcoxon signed rank testi ve Spearman's rank testi kullanıldı.

Bulgular: 10 µg/ml fitohemagglütinin (PHA) ile inkübe edilen kültür süpernatanlarında IL-10 ve IL-13 düzeyleri hastalar ve sağlıklı kontrollerde benzer bulundu ($P > 0.05$). 20 µg/ml PHA ile inkübe edilen kültür süpernatanlarında IL-10 düzeyleri tip-2 DM 'lü hastalarda kontrol grubuna göre anlamlı düşük bulundu (sırasıyla, kötü metablik kontrol için $P = 0.001$ ve iyi metablik kontrol için $P = 0.048$).

Sonuç: Bu çalışmaya göre IL-13'deki değişiklikler tip-2 DM ve tip-1 DM patogenezinde önemli bir rol oynamamaktadır. Ancak diyabetli hastaların yüksek dozda PHA ile uyarılan periferik kan mononükleer hücrelerinden üretilen IL-10'un düşük bulunması tip-2 DM patogenezinde rolü olabileceğini düşündürmektedir.

Anahtar Sözcükler: IL-10, IL-13, Diabetes Mellitus

Received: November 22, 2006
Accepted: January 29, 2007

Correspondence
Göksal KESKİN
Güvenlik Cad. 123/2
06690 A. Ayrancı,
Ankara - TURKEY
EDC85@turk.net

Introduction

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia, which is accompanied by lipid metabolic disorders. There are two types: non-insulin-dependent diabetes mellitus (type-2 DM) and insulin-dependent diabetes mellitus (type-1 DM). Two major pathogenetic mechanisms are operative in type-2 DM. One of them is impaired islet beta (B) cell function and the other is impaired insulin action. Recently it has been reported that impaired cell-mediated immunity was seen in patients with type-2 DM (1). Insulin-dependent DM is an organ-specific chronic autoimmune disease resulting from T-cell-mediated destruction of pancreatic cells. It is generally accepted that proinflammatory cytokines secreted by mononuclear cells are responsible for pancreatic B cell destruction in animal models of autoimmune type-1 DM (2). In type-1 DM, the infiltration of the islets of the pancreas with mononuclear cells leads to the damage of B cells and causes insulin deficiency. Several studies have shown that markers of inflammation and pro-inflammatory cytokines such as interleukin (IL)-10 and IL-13 associate with the metabolic syndrome, dyslipidemia, type-2 DM and type-1 DM (3-5).

Interleukin-10 is produced by activated Th2 lymphocytes, mast cells, monocytes, B lymphocytes, eosinophils and keratinocytes (6). IL-10 is also produced by activated human natural killer cells and has been identified as a vital modulator of abundant production of inflammatory cytokines (7). It acts to inhibit proinflammatory cytokines, growth factors and chemokine production by mononuclear phagocytes (6,8). It down-regulates various inflammatory events. Monocytes pretreated with IL-10 fail to induce antigen-specific T-cell proliferation (6). In an IL-10 transgenic model, IL-10 enhanced the accumulation of CD8 T cells in the pancreas leading to an earlier onset of diabetes in nonobese diabetic mice (9).

Interleukin-13 is a Th2 cytokine with many of the same activities as IL-4 because they share some of the same surface receptors. IL-13 can suppress the cytotoxic functions of monocytes-macrophages and inhibit the production of macrophage-derived proinflammatory cytokines (10). The data on IL-13 are variable. Kretowski et al.'s (2) findings suggest that IL-13 alterations could play an important role both in the pathogenesis and in the prevention of type-1 DM. It was suggested the IL-13 levels correlate with the activity of the autoimmune

process (11). Peripheral blood mononuclear cells (PBMCs) from patients with type-2 DM show reduced proliferative response to phytohemagglutinin (PHA) and other mitogens (1).

The aim of this study was to examine the in vitro production of IL-10 and IL-13 in cultures of peripheral lymphocytes obtained from patients with type-2 DM, type-1 DM and healthy controls.

Materials and Methods

All patients with DM were diagnosed in accordance with the Report of the Expert Committee on the Diagnosis and Classification of Diabetes (12). Twenty patients (10 females, 10 males) with type-2 DM (11 patients with poor metabolic control, HbA1C>7.5%, median age: 57 years, and median disease duration:10 years; 9 patients with good metabolic control, HbA1C<7.5%, median age: 63 years, and median disease duration: 11 years), 10 patients (5 females, 5 males) with type-1 DM (median age: 24 years and median disease duration: 11 years) and the control group were enrolled in the study. The control group included 10 healthy subjects (5 females and 5 males, median age: 57 years) matched for age, sex and body mass index (BMI) with type-2 DM patients, but with no clinical or laboratory signs of any disease. The demographic and descriptive details of all patients and controls are shown in Table 1.

Table 1. Demographic characteristics of patients with NIDDM and IDDM and healthy controls.

	Patients [‡] (n = 11)	Patients [†] (n = 9)	Patients [§] (n = 10)	Controls (n = 10)
Age (year)	57 (27)	63 (31)	24 (15)	57 (33)
Sex (M/F)	6/5	4/5	7/4	5/5
BMI (kg/m ²)	28 (10)	28 (11)	26 (13)	26 (11)
Disease duration	10 (12)	11 (12)	11 (14)	-

Values are given as Median (Range).
 NIDDM: Non-insulin-dependent diabetes mellitus. IDDM: Insulin-dependent diabetes mellitus. M: Male. F: Female. BMI: Body mass index.
 NS: Not significant.
[‡] Patients with NIDDM (poor metabolic control).
[†] Patients with NIDDM (good metabolic control).
[§] Patients with IDDM.

Twelve patients with type-2 DM had hypertension and had taken antihypertensive drugs. Ten patients had hyperlipidemia. All of the type-2 DM patients had taken oral antidiabetic drugs (OAD). All of the type-1 DM patients had taken insulin.

Exclusion criteria included abnormal laboratory test results such as the levels of serum urea, creatinine, liver enzymes, erythrocyte sedimentation rate, C-reactive protein, white and red blood cell counts, and thrombocytes. Cases were also excluded from the trial if they had any autoimmune or infectious diseases. Informed consent was obtained from all patients and healthy controls. The study protocol was approved by the Ethics Review Committee of the Medical School of Gulhane.

Cell preparations and cultures: PBMCs were isolated from 20 ml of heparinized venous blood by centrifugation on Ficoll-Hypaque Separating Solution (Seromed[®], Biochrom KG Berlin, Germany). Then, PBMCs were washed twice in sterile phosphate buffered saline (PBS) and resuspended in RPMI-1640 medium (Gibco, Invitrogen Corporation, Scotland, UK) supplemented with 10% heat inactivated newborn calf serum (NCS) (Gibco, Invitrogen Corporation, Scotland, UK). These cell suspensions were incubated in culture flasks (Nunc Brand Products, Roskilde, Denmark) at 37 °C in a humidified 5% CO₂ atmosphere for 2 h. Adherent cells were discarded, but nonadherent cells were collected from upper layers of the cell suspensions, washed twice in PBS, and resuspended in RPMI-1640 medium supplemented with 10% NCS, 100 IU/ml penicillin, 10 m/ml streptomycin (Sigma Chemical Co. St Louis, MO, USA), and 2 mM/ml L-glutamine (Gibco, Invitrogen Corporation, Scotland, UK) for cell culture. Viability in the cell preparations was determined by acridine-orange staining method, and found as more than 95%.

Peripheral blood mononuclear cells were cultured at a concentration of 1×10^6 /ml in RPMI-1640 medium supplemented with 10% NCS, 100 IU/ml penicillin, 100 m/ml streptomycin, and 2 mM/ml L-glutamine. Cells were cultured in 24 well plates (Nunc Brand Products, Roskilde, Denmark) in the absence or presence of phytohemagglutinin-M (PHA-M, Gibco, Invitrogen Corporation, Scotland, UK) at 37 °C in a humidified 5% CO₂ atmosphere for 36 h. PHA-M was used at final

concentrations of 10 mg/ml and 20 mg/ml in the cultures. Culture supernatants were collected at the end of incubation, then centrifuged at 550g for 5 min and stored at -70 °C until IL-10 and IL-13 analyses.

Measurement of IL-10 and IL-13: IL-10 and IL-13 in culture supernatants were measured by the enzyme immunoassay method using commercially available ELISA kits (Cytimmune Sciences Inc, College Park, MD, USA). According to kit prescription, sensitivity was 1.6 pg/ml and 7.6 pg/ml, intraassay variation $\pm 8.4\%$ for both, and interassay variation $\pm 10.2\%$ and $\pm 11.9\%$ for IL-10 and IL-13, respectively.

Statistical analysis: Statistical analyses were performed using Mann-Whitney U test, Wilcoxon signed rank tests, and Spearman's rank correlation in the SPSS for Windows 11.5 statistical software (SPSS Inc. Chicago, IL, USA). A value of $p \leq 0.05$ was considered to represent a significant difference between analyzed data.

Results

The mean IL-10 and IL-13 levels in culture supernatants incubated with 10 μ g/ml PHA were 199 ± 75 pg/ml and 113 ± 31 pg/ml in type-2 DM patients, 209 ± 38 pg/ml and 101 ± 15 pg/ml in type-1 DM patients, and 228 ± 35 pg/ml and 123 ± 42 pg/ml in healthy controls, respectively. The mean IL-10 and IL-13 levels in culture supernatants incubated with 20 μ g/ml PHA were 286 ± 38 pg/ml and 185 ± 67 pg/ml in type-2 DM patients, 306 ± 44 pg/ml and 182 ± 24 pg/ml in type-1 DM patients, and 448 ± 166 pg/ml and 225 ± 78 pg/ml in the healthy controls, respectively (Table 2).

In all groups, IL-10 and IL-13 levels were significantly high in culture supernatants incubated with 20 μ g/ml PHA compared to levels in culture supernatants incubated with 10 μ g/ml PHA ($P = 0.002$ and $P = 0.001$ in the patients, $P = 0.005$ and $P = 0.005$ in the controls, respectively).

IL-10 and IL-13 levels in all patients and healthy controls were similar in culture supernatants incubated with 10 μ g/ml PHA ($P < 0.05$). IL-13 levels in controls were higher than in the patients in culture supernatants incubated with 20 μ g/ml PHA, but without statistical significance. IL-10 levels in type-2 DM patients were

Table 2. Comparisons of IL-10 and IL-13 production by PBMCs and some metabolic parameters in patients with NIDDM and IDDM and healthy controls.

	Patients [§] (n = 11)	Patients [†] (n = 9)	Patients [‡] (n = 10)	Controls (n = 10)	χ ²	P
IL-10* (pg/ml)	203 (317)	185 (196)	235 (149)	221 (120)	2.383	0.497
IL-10** (pg/ml)	268 (241)	279 (578)	321 (627)	434 (568)	8.720	0.033
IL-13* (pg/ml)	108 (111)	96 (92)	105 (109)	106 (133)	1.001	0.801
IL-13** (pg/ml)	200 (173)	187 (238)	208 (221)	219 (215)	2.229	0.526
HbA1C (%)	8.7 (1.9)a	6.8 (1.4)b	7.0 (3.8)c	6.1 (2.1)d	23.195	<0.001
Glucose (mg/dl)	160 (111)e	128 (103)f	121 (120)	101 (44)g	13.669	0.003
Cholesterol (mg/dl)	208 (109)	186 (72)	199 (75)	192 (93)	1.934	0.586
Triglyceride (mg/dl)	208 (262)	163 (103)	163 (175)	166 (178)	1.655	0.647
HDL (mg/dl)	42 (22)	46 (23)	39 (21)	45 (31)	1.564	0.668

Descriptive statistics are presented as median (range). For multiple groups, we used Kruskal-Wallis test. The differences between two groups were evaluated by Wilcoxon signed rank tests or Mann-Whitney U test, where appropriate.

PBMC: Peripheral blood mononuclear cells. NIDDM: Non-insulin-dependent diabetes mellitus. IDDM: Insulin-dependent diabetes mellitus. HDL: High density lipoprotein.

* IL-10 and IL-13 levels in culture incubated with 10 mg/ml phytoagglutinin (PHA).

** IL-10 and IL-13 levels in cultures incubated with 20 mg/ml PHA.

[§] Patients with NIDDM (poor metabolic control).

[†] Patients with NIDDM (good metabolic control).

[‡] Patients with IDDM.

P < 0.05 for * vs **, in all study groups; P < 0.001 for a vs b, a vs d,

P = 0.004 a vs c, P = 0.001 e vs g and P = 0.021 f vs g.

significantly lower than in the control group in culture supernatants incubated with 20 µg/ml PHA (P = 0.001 for patients with poor metabolic control and P = 0.048 for patients with good metabolic control, respectively) (Figure 1.). When we compared IL-10 and IL-13 levels in patients with good and poor metabolic control, there was no statistically significant difference.

Discussion

Impaired cell-mediated immunity in diabetics is being reported with increasing frequency. The results of several studies have suggested important roles of cytokines in the pathogenesis of both type-2 DM and type-1 DM (2,5,13,14). It has been reported that long-standing metabolic abnormalities might affect the monocyte function (13). We detected that good and poor metabolic control did not affect IL-10 and IL-13 production by peripheral blood lymphocytes in patients with type-2 DM

and type-1 DM. Ragab et al. (15) found no differences between normal individuals and diabetic individuals with good metabolic control when they compared the responses of peripheral blood lymphocytes to either PHA. We also found similar results in our study.

The infiltration of the islets of the pancreas with mononuclear cells leads to the damage of B cells and causes insulin deficiency. In an IL-10 transgenic model, IL-10 enhanced the accumulation of CD8 T cells in the pancreas leading to an earlier onset of diabetes in nonobese diabetic mice (16). IL-10 production in vitro by lymphocytes from high-risk of type-1 DM patients was spontaneously enhanced both in nonstimulated and insulin-stimulated cultures (14). PHA increased interferon (IFN) gamma, IL-6 and IL-10 production in all groups (14). It is postulated that IL-10 favors the activation of the antigen-presenting cell population and down-regulates the autoreactive effector cells at the site of the immune reaction (9). Tchorzewski (14) observed the

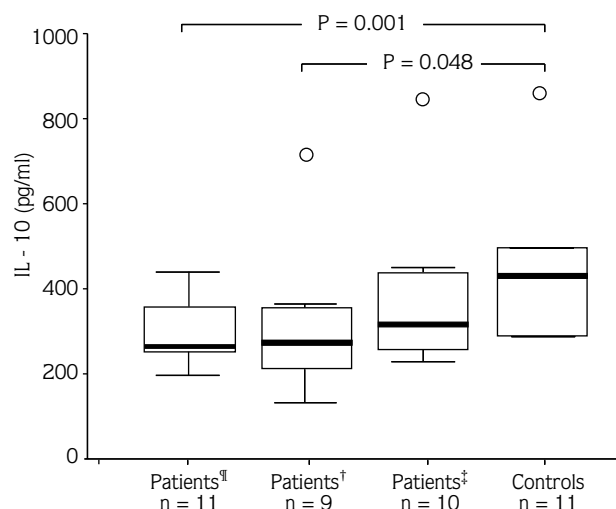


Figure 1. Comparisons of IL-10 production in the cultures of patients and controls induced by 20 mg/ml PHA-M. Boxes show the ranges of 1st and 3rd quartiles and extreme values, with the horizontal bars representing median values. The differences between two groups were evaluated by Mann-Whitney U test. p values were indicated above the boxes when a level of significance < 0.05 was reached in comparisons of study groups.

¶ Patients with NIDDM (poor metabolic control).

† Patients with NIDDM (good metabolic control).

‡ Patients with IDDM.

increase in IL-10 production by lymphocytes of DM patients with high risk, which may implicate the autocrine role of this cytokine in the pathogenesis of islet cells destruction and type-1 diabetes development. In our study, in type-1 DM, type-2 DM and healthy controls, IL-10 levels were similar in culture supernatants incubated

with 10 µg/ml PHA, but IL-10 production was significantly higher in culture supernatants incubated with 20 µg/ml in healthy controls compared to levels in the patients with type-2 DM and type-1 DM.

It has previously been shown that IL-13, a recently discovered cytokine, can suppress the cytotoxic function of monocytes/macrophages, inhibit the production of macrophage-derived proinflammatory cytokines, and have a modulatory effect on the B lymphocytes function (17-20). The main anti-inflammatory properties of IL-13 are associated with the downregulation of the secretion of IL-1beta and tumor necrosis factor (TNF) alpha by macrophages/monocytes (2,17). The alteration in IL-13 is also believed to contribute to the development of autoimmune diseases (21-23). In our study, IL-13 production was similar with 10 and 20 µg/ml PHA-stimulated peripheral blood lymphocytes in all groups. We also studied IL-10 and IL-13 production of PBMCs in patients with type-2 DM.

It has been reported that PBMCs from patients with type-2 DM show reduced proliferative response to PHA and other mitogens (1,24). In our study, the proliferative response of IL-10 with 10 µg/ml PHA remained unchanged, but the response was also not enough with 20 µg/ml PHA.

According to our findings, the alterations in IL-13 did not play an important role in pathogenesis of type-2 DM and type-1 DM. Furthermore, the deficiency in IL-10 production from PBMCs with high dosages of PHA suggests that IL-10 may play an important role in the pathogenesis of type-2 DM.

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