Immunobiology 218 (2013) 1113–1118

Contents lists available at SciVerse ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Therapeutic improvement of glucoregulation in newly diagnosed type 2 diabetes patients is associated with a reduction of IL-17 levels

Mirjana Sumarac-Dumanovic[a], Danka Jeremic[a], Aleksandar Pantovic[b], Kristina Janjetovic[b], Danica Stamenkovic-Pejkovic[a], Goran Cvijovic[a], Darko Stevanovic[c], Dragan Micic[a,*], Vladimir Trajkovic[b,**]

[a] Clinic for Endocrinology, Diabetes and Diseases of Metabolism, School of Medicine, University of Belgrade, Dr Subotica 13, Belgrade 11000, Serbia
[b] Institute of Microbiology and Immunology, School of Medicine, University of Belgrade, Dr Subotica 1, Belgrade 11000, Serbia
[c] Institute of Medical Physiology, School of Medicine, University of Belgrade, Visegradska 26, Belgrade 11000, Serbia

ARTICLE INFO

Article history:
Received 31 August 2012
Received in revised form 7 February 2013
Accepted 11 March 2013
Available online 16 March 2013

Keywords:
Diabetes type 2
Glycated hemoglobin
Metformin
Th1
Th17

ABSTRACT

We explored the effect of therapeutic glucoregulation on the blood levels of proinflammatory T helper (Th)17 cytokines interleukin (IL)-17 and IL-23, and Th1 cytokines interferon (IFN)-γ and IL-12 in newly diagnosed type 2 diabetes patients. The investigated group consisted of 23 subjects (17 men and 6 women, age 26–64). The cytokine serum levels, glycated hemoglobin (HbA1c) as a marker of glucoregulation, homeostasis model assessment index as a measure of insulin resistance (HOMA-IR), and body mass index (BMI) were determined before and after 12 weeks of therapy consisting of standard lifestyle modification and metformin (1000 mg b.i.d.). The levels of Th17 and Th1 cytokines before treatment did not correlate with age, BMI or HOMA-IR. The patients with poor glucoregulation (HbA1c > 7%, $n = 12$), compared to those with good glucoregulation (HbA1c ≤ 7%, $n = 11$), had higher serum levels of Th17 and Th1 cytokines, but only the differences in IL-17 (median 21.2 pg/ml vs. 4.8 pg/ml) and IFN-γ 5 (0.6 pg/ml vs. 27.7 pg/ml) reached statistical significance ($p = 0.003$ and $p = 0.012$, respectively). The reduction of HbA1c values (from 8.6 to 5.9%, $p = 0.000$) observed upon treatment in patients with poor glucoregulation was associated with a significant decrease in the concentration of IL-17 (from 21.2 to 12.9 pg/ml, $p = 0.020$), but not IFN-γ (50.6 vs. 52.3, $p = 0.349$). These data indicate that therapeutic improvement of glucoregulation might contribute to a reduction of IL-17 levels in newly diagnosed type 2 diabetes patients.

Introduction

It has been well documented that type 2 diabetes (T2D) and metabolic syndrome are associated with low-grade systemic inflammation (Donath et al. 2009; Pickup 2004; Shoelson et al. 2006; Sjoholm and Nystrom 2006). A number of studies have described elevated circulating levels of acute-phase proteins such as C-reactive protein, haptoglobin, fibrinogen, plasminogen activator inhibitor and serum amyloid A, as well as various proinflammatory cytokines in patients with T2D (Festa et al. 2002; Pickup et al. 1997; Spranger et al. 2003; Tan et al. 2004). The immune cell activation with the subsequent increase in circulating inflammatory marker levels has been regarded both as a consequence of metabolic disturbances in T2D (Greenfield and Campbell 2006) as well as a positive feedback mechanism involved in their progression (Donath et al. 2009; Pickup 2004; Sjoholm and Nystrom 2006).

Interleukin (IL)-17-producing T helper (Th)17 cells are induced by several cytokines, including IL-23, and represent a major helper T cell subset involved in the pathogenesis of various autoimmune/inflammatory diseases, including multiple sclerosis, rheumatoid arthritis and psoriasis (Gaffen 2011; Korn et al. 2009). IL-17 promotes inflammation through a widely expressed family of IL-17 receptors, many of which trigger intracellular signaling linked to nuclear factor-κB (NF-κB) activation, thus leading to proinflammatory cytokine production by monocytes, fibroblast, stromal, epithelial, and endothelial cells (Gaffen 2009; Miljkovic and Trajkovic 2004). IL-17 also mobilizes and recruits granulocytes through induction of granulopoeisis and CXC chemokine production, as well as increased local survival (Kolls and Linden 2004). Similarly to Th17 response, IL-12-differentiated Th1 cells producing IFN-γ have been implicated in organ-specific autoimmunity, which

Abbreviations: T2D, type 2 diabetes; CRP, C-reactive protein; TNF, tumor necrosis factor; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated hemoglobin; AMPK, AMP-activated protein kinase; NF-κB, nuclear factor-κB.

* Corresponding author. Tel.: +381 11 656 527; fax: +381 11 3065 081.
** Corresponding author. Tel.: +381 11 3643 233; fax: +381 11 3643 235.
E-mail addresses: micicd@eunet.rs (D. Micic), vtrajkovic@med.bg.ac.rs (V. Trajkovic).

0171-2985/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.imbio.2013.03.002

is characterized by the presence of Th1 cells and IFN-γ-activated macrophages at the sites of inflammation (Dardalhon et al. 2008). The inflammation and insulin resistance in T2D are associated with an expansion of both Th17 and Th1 subsets and a decrease in the T regulatory subset, indicating a potential beneficial effect of the therapies targeted toward resetting this balance (Jagannathan-Bogdan et al. 2011). Although various therapeutic approaches, including lifestyle modification and antihyperglycemic agents such as insulin or metformin, have been shown to reduce circulating levels of CRP, IL-6 or tumor necrosis factor (TNF) in T2D patients (Aas et al. 2006; Belalcazar et al. 2010; Carter et al. 2005; Fidan et al. 2011; Noh et al. 2008; Takebayashi et al. 2004), the effect of antidiabetic therapy on Th17 and Th1 cytokines has not been extensively studied.

The aim of the present study was to investigate the activity of IL-23/IL-17 and IL-12/IFN-γ cytokine axis in newly diagnosed T2D patients before and after three months of therapy consisting of lifestyle modification and metformin.

Materials and methods

Subjects

The investigated group consisted of 23 subjects (17 men and 6 women) newly diagnosed with prediabetes or type 2 diabetes (American Diabetes Association 2010). The patients were followed during period of 12 weeks on metformin therapy (Glucophage®, Merck Serono, 1000 mg b.i.d.) and standard lifestyle modification consisting of diet and exercise. The study inclusion/exclusion criteria were as follows: drug-naïve newly diagnosed diabetic patients of both sexes under 65 years of age, no smoking habits, no alcohol consumption, normal thyroid function, blood pressure 130/80 mm Hg or less on antihypertensive therapy, and no other drugs including hypolipemic drugs. The study was conducted after obtaining informed consent from all subjects. The study was approved by the Ethics Committee of the School of Medicine Belgrade.

Determination of anthropometric and biochemical parameters

The body mass index (BMI) was calculated by dividing weight (kg) by the squared value of height in meters. Samples of venous blood were collected in the morning, after an overnight fast, and the serum was obtained by centrifugation. The serum samples were stored at −70 °C. Insulin resistance was determined based on the homeostasis model assessment index (HOMA-IR), calculated from the fasting glucose and insulin concentrations: HOMA-IR = fasting insulin (IU/ml) – fasting glucose (mmol/l)/22.5. Insulin was determined by radioimmunoassay (RIA) kit (INEP Zemun, Belgrade, Serbia). Glycated hemoglobin (HbA1c), as a measure of average serum glucose concentration, was determined by the VARIANT II Hemoglobin A_{1C} Programm based on HPLC method. The HbA1c of 7% as a cut-off value for good glucose control (American Diabetes Association 2010) was used to create two subgroups, $HbA1c^{high}$ (HbA1c > 7%, $n = 12$) and $HbA1c^{low}$ (HbA1c ≤ 7%, $n = 11$).

Table 1
The effect of treatment on the main clinical/biochemical parameters and Th1/Th17 cytokine levels in newly diagnosed patients with T2D. The data are median values with interquartile range in parentheses.

	Before	After	*p*
Age (years)	51 (13)	n.a.	n.a.
HbA1c (%)	7.4 (2.1)	6.0 (0.9)	0.000* (0.007)
HOMA-IR	8.4 (9.4)	4.5 (2.6)	0.018 (0.090)
BMI (kg/m^2)	30.2 (8.8)	28.1 (6.8)	0.000* (0.007)
IL-17 (pg/ml)	11.3 (19.6)	11.3 (9.8)	0.269
IL-23 (pg/ml)	5.1 (6.0)	4.1 (5.1)	0.082
IFN-γ (pg/ml)	44.1 (26.2)	31.0 (45.9)	0.125
IL-12 (pg/ml)	4.8 (14.4)	4.8 (6.0)	0.191

n.a., not applicable.
* Statistically significant difference with Holm-Bonferroni adjusted *p* in parentheses.

Cytokine measurements

Serum concentrations of cytokines were measured by commercial ELISA kits (eBioscience, San Diego, CA). Lower limit of detection was 4 pg/ml, as reported by the manufacturer (IL-17, IL-12, IFN-γ) or determined in our laboratory (IL-23). The inter- and intra-assay coefficients of variability for all cytokines were <15% and <10%, respectively.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences version 17 and the *p* values less than 0.05 were considered significant. For a desired value of $p < 0.05$ and 80% power to detect an actual difference, a sample size of 23 was considered satisfactory. The Shapiro–Wilks test revealed that the data for most of the variables were not normally distributed. Therefore, Mann–Whitney or Wilcoxon matched-pairs signed-rank test was used to assess the significance of the difference in cytokine levels between different groups or in the same group before and after metformin treatment, respectively. The detection frequencies of cytokines were compared using Chi-square test, while Spearman correlation analysis was employed to assess the correlation between different parameters. The obtained *p* values were corrected for multiple testing using Holm-Bonferroni correction.

Results

The values of main clinical/biochemical parameters and cytokine concentrations assessed before and after treatment with metformin are presented in Table 1. In comparison with the baseline values, newly diagnosed diabetic patients after the therapy had significantly lower HbA1c and BMI values, while the initially significant difference in HOMA-IR index disappeared after correcting for multiple testing. On the other hand, the levels of IL-17, IL-23, IFN-γ and IL-12 were clearly not significantly different before and after the treatment (Table 1).

While the concentrations of Th17 and Th1 cytokines at baseline were not correlated with age, BMI or HOMA-IR (Table 2), the levels of IL-17, IL-23 and IL-12 initially displayed a significant positive correlation with the values of HbA1c (Fig. 1). Although the

Table 2
Correlation between Th17/Th1 cytokine levels and clinical/biochemical parameters in T2D patients. The Spearman correlation coefficient is presented with the corresponding *p* values in parentheses.

	IL-17	IL-23	IFN-γ	IL-12
Age	−0.195 (0.372)	−0.263 (0.225)	−0.246 (0.257)	−0.157 (0.474)
HOMA-IR	0.273 (0.207)	0.048 (0.828)	0.063 (0.774)	0.112 (0.612)
BMI (kg/m^2)	0.057 (0.797)	−0.094 (0.670)	−0.148 (0.501)	−0.064 (0.771)