

SERUM LEVELS OF INTERLEUKIN 12 AND INTERLEUKIN 18 IN DIABETIC CHILDREN

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SUMMARY

Forty-six children with type 1 diabetes mellitus (mean age 13.19 ± 3.5 years) and 10 healthy age- and sex-matched volunteers (13.6 ± 4.2 years) were included in the study to compare serum levels of interleukin (IL)-18 and IL-12. IL-18 and IL-12 in serum were measured using an enzyme-linked immunosorbent assay (ELISA). Diabetic subjects were divided into two groups according to the presence (group 1) or absence (group 2) of vascular complications. Significant differences were observed in serum levels of IL-18 and IL-12 between diabetic patients and control subjects (IL-18: 705.6 ± 515.3 pg/mL vs. 128.1 ± 80.7 pg/mL; $p=0.0009$; and IL-12: 245.6 ± 100 pg/mL vs. 58.5 ± 17.3 pg/mL; $p=0.0001$). Group 1 showed a non-significantly higher level of IL-12 than group 2 ($p=0.078$). There was no significant difference in serum IL-18 between group 1 and group 2 ($p=0.28$). In patients with vascular complications, there was a correlation between serum IL-12 and age ($r=0.48$; $p=0.046$). In conclusion, elevated levels of IL-18 and IL-12 suggest that both interleukins may have some etiopathogenic role in diabetes mellitus in children.

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INTRODUCTION

Type 1 (insulin dependent) diabetes mellitus (T1DM) is an autoimmune disease with both genetic and environmental components. Interleukin-12 p40 production influences T cell response, and may therefore be important in T1DM pathogenesis (1). Interleukin-12 (IL-12) drives the differentiation of T lymphocytes towards the Th1 subset, characterized by production of cytokines leading to cell mediated immunity (2-4).

In addition, IL-12 is important in immune response to infections; however, it has been shown that in the absence of infection, IL-12 induced autoreactive T cell responses might predispose to self-destructive immunity (5,6). IL-12 is a disulfide linked heterodimer composed of a heavy chain of 40 kDa (p40) and a light chain of 35 kDa (p35). The two subunits of IL-12 are in contrast to most cytokines, which possess only one polypeptide chain. Many cell types express the p35 chain, while the p40 subunit is expressed mainly by activated macrophages and B cells (7). The heterodimers, p70 or p75, are biologically active parts of IL-12 (8).

IL-18, also known as interferon-gamma (IFN- γ) inducing factor, is a cytokine produced by antigen presenting cells. IL-18 exerts several effects on Th1 cells. IL-18 stimulates Th1 cell proliferation, and also

works in combination with IL-12 to induce the production of IFN- γ , GM-CSF, and IL-2 by Th1-type cells.

Macrophages and T lymphocytes are the first cells to appear in pancreatic islets in the development of autoimmune diabetes. It has been suggested that cytokines released by monocytes/macrophages, including IL-1 β , IL-12 and tumor necrosis factor- α (TNF- α) could have an initial role in islet B-cell damage. The aim of the present study was to estimate the levels of IL-12 and IL-18 in 46 diabetic children and 10 healthy controls.

MATERIALS AND METHODS

Subjects

The baseline study population consisted of 46 patients (21 boys and 25 girls) with type 1 diabetes mellitus (mean age 13.19 ± 3.5 years) diagnosed according to the World Health Organization definition, and a control group of 10 age- and sex-matched healthy children (13.6 ± 4.2) with no family history of diabetes. Diabetic children were residing in the vicinity of the Pleven University Hospital and were treated with a standard dose of human insulin obtained from Novo Nordisk Industry, Copenhagen, Denmark.

The mean duration of diabetes was 5.9 ± 3.9 years. All patients were taking 2 to 4 subcutaneous insulin doses *per* day. Diabetics were divided into two groups according to the presence (group 1) or absence (group 2) of vascular complications.

Ethical approval was obtained from the local research ethics committee and parents of all subjects gave an informed written consent prior to the study.

Procedure

The enzyme-linked immunosorbent assay (ELISA) for IL-12 was performed according to the protocol developed by BioSource, Europe S.A. (BioSource IL-12+p40 EASIA kit) and for IL-18 by BioSource, Europe S.A. (BioSource human interleukin-18 ELISA kit).

Statistics

All values were expressed as mean \pm SD. Statistical analyses were done using the Excel and Statgraphics Plus for Windows softwares. Student's t-test and ANOVA were used to assess differences between study groups. Correlation analysis was also performed. The level of significance was set at $p < 0.05$.

RESULTS

Diabetic patients were divided into two groups: group 1 ($n=17$) and group 2 ($n=29$). Clinical data of the patients are presented in Table 1. The cutoff point for positivity was set at mean+2 SD (i.e. 2 SD above the mean in healthy subjects in each age group).

Table 1. Clinical characteristics of diabetic patients with or without vascular complications

Clinical data	Group 1	Group 2	p
Age (yrs)	12.8 \pm 3.8	13.7 \pm 2.9	0.373
Duration (yrs)	5.3 \pm 3.9	4.7 \pm 3.9	0.601
Dose (U/kg/24 h)	0.985 \pm 0.295	1.00 \pm 0.24	0.813
Mean glycated hemoglobin (%)	10.71 \pm 2.93	9.17 \pm 1.37	0.043
Total cholesterol (mmol/L)	4.43 \pm 1.14	4.98 \pm 1.76	0.199
High-density lipoprotein (mmol/L)	1.53 \pm 0.58	1.48 \pm 0.36	0.732
Triglycerides (mmol/L)	1.25 \pm 0.91	1.66 \pm 1.63	0.275
Microalbuminuria (μ g/min)	35.03 \pm 54.22	15.05 \pm 6.27	0.128
Systolic blood pressure (mm Hg)	110.17 \pm 13.77	112.77 \pm 9.58	0.488
Diastolic blood pressure (mm Hg)	73.75 \pm 8.9	73 \pm 7.09	0.783
IL12pg (pg/mL)	266.4 \pm 112.1	213.2 \pm 68.5	0.078
IL18pg (pg/mL)	772.8 \pm 463.1	604.8 \pm 584.3	0.289

Group 1, patients with vascular complications ($n=17$); group 2, patients without vascular complications ($n=29$)

Diabetics showed a statistically significantly higher level of IL-12 than controls (245.6 ± 100 pg/mL *vs.* 58.5 ± 17.3 pg/mL; $p=0.0001$). Group 1 showed a non-significantly higher level of IL-12 than group 2 ($p=0.078$)

The results obtained by ELISA for IL-18 were similar to those on IL-12. Diabetics had a significantly higher level than controls (705.6 ± 515.3 pg/mL *vs.*

128.1±80.7 pg/mL; $p=0.0009$). There were no significant differences between group 1 and group 2 ($p=0.28$).

In patients without vascular complications the level of IL-12 correlated with age ($r=0.48$; $p=0.046$).

There was no correlation between the study interleukins and other parameters.

DISCUSSION

The precise mechanisms by which IL-12 and IL-18 could induce pathological damage in diabetics have not been completely clarified. We tried to obtain sufficient evidence to prove the association between the existence of these cytokines in diabetic patients and the disease. The significance of IL-12 in human autoimmunity is not clear, and serum levels of IL-12 in diabetes mellitus have not been well established. Elevated levels of this cytokine have been observed in most autoimmune diseases (9,10). The development of type 1 diabetes in animal models is T cell dependent. Islet inflammation begins as peripheral benign Th2 type insulinitis and progresses to destructive Th1 type insulinitis, which is driven by the innate immune system *via* secretion of IL-12 and IL-18 (10). We found higher cytokine levels of IL-12 and IL-18 in the patients as compared with the control group. Mojtahedi *et al.* (11) found an association between single-nucleotide polymorphisms in the promoter region of IL-18 gene and type 1 diabetes. Their results have shown that polymorphisms of IL-18 promoter confer susceptibility to type 1 diabetes with the onset at an older age. In our study we found a correlation between age and IL-12. IL-18 works in combination with IL-12 to induce production of cytokine by Th1-type cells, but no correlation was found with age.

REFERENCES

1. Adorini L. Interleukin 12 and autoimmune diabetes. *Nature Genet* 2001;27:131-132.
2. Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 CD4(+)T-cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 1995;16:34-38.
3. Tian J, Olcott AP, Hanssen LR, Zekzer D, Middleton B, Kaufman DL. Infectious Th1 and Th2 autoimmunity in diabetes-prone mice. *Immunol Rev* 1998;164:119-127.
4. Manetti R, Parronchi P, Giudizi MG, Piccinni MP, Maggi E, Trinchieri G, Romagnani S. Natural-killer-

Esposito *et al.* (12) have recently reported that IL-18 and other proinflammatory cytokines are increased by hyperglycemia in subjects with impaired glucose tolerance, suggesting a causal role for hyperglycemia in the immune activation of diabetes. We confirm this result, because all diabetics had elevated serum level of IL-18. However, they conclude that IL-18 is stimulated by stress hyperglycemia and could play a role in acute coronary syndromes. We found no relation between microvascular complications and IL-18. It is well known that subjects with type 1 diabetes have a 2- to 4-fold risk of death from coronary heart disease in comparison with nondiabetic age-matched individuals, and hyperglycemia is believed to be a key risk factor in the development of micro- and macrovascular complications (13). Perhaps the absence of correlation could be explained by age differences between these two studies. It is difficult to compare diabetic children and adult patients, when many different factors are present.

Kretowski *et al.* (13,14) observed that in newly diagnosed subjects with type 1 diabetes, fasting baseline serum levels of IL-18 were increased in comparison with healthy controls, and the degree of these elevations did not depend on the level of glycemic control (HbA_{1c}). We found no correlation between diabetic microvascular complications and IL-12 and/or IL-18, probably due to the small study group, which did not allow for any definite conclusions to make. That is why a more representative population should be investigated in some future studies.

In the present study, we confirmed the elevation of IL-12 and IL-18 in type 1 diabetes. Future investigations including other cytokines and gene polymorphism will help clarify the role of interleukins in the etiopathogenesis of Th1 dependent diseases such as diabetes.

- cell stimulatory factor (interleukin-12 (IL-12)) induces T-helper type-1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th2 cells. *J Exp Med* 1993;177:1199-1204.
5. Segal BM, Shevach EM. IL-12 unmasks latent autoimmune disease in resistant mice. *J Exp Med* 1996;184:771-775.
 6. Segal BM, Klinman DM, Shevach EM. Microbial products induce autoimmune disease by an IL-12-dependent pathway. *J Immunol* 1997;158:5087-5090.
 7. Huang D, Cancilla MR, Morahan G. Complete primary structure, chromosomal localisation, and definition of polymorphisms of the gene encoding the human interleukin-12 p40 subunit. *Genes Immun* 2000;1:515-520.
 8. Mattner F, Fischer S, Guckes S, Jin SC, Kaulen H, Schmitt E, Rude E, Germann T. The interleukin-12 subunit p40 specifically inhibits effects of the interleukin-12 heterodimer. *Eur J Immunol* 1993;23:2202-2208.
 9. Tokano Y, Morimoto S, Kaneko H, Amano H, Nozawa K, Takasaki Y, Hashimoto H. Levels of IL-12 in the sera of patients with systemic lupus erythematosus (SLE) – relation to Th1- and Th2-derived cytokines. *Clin Exp Immunol* 1999;116:169-173.
 10. Rothe H, Hausmann A, Casteels K, Okamura H, Kurimoto M, Burkart V, Mathieu C, Kolb H. IL-18 inhibits diabetes development in nonobese diabetic mice by counter-regulation of Th1-dependent destructive insulinitis. *Immunology* 1999;116:1230-1236.
 11. Mojtahedi Z, Naeimi S, Farjadian S, Omrani GR, Ghaderi A. Association of IL-18 promoter polymorphisms with predisposition to type 1 diabetes. *Diabet Med* 2006;23:235-239.
 12. Esposito K, Nappo F, Marfella R, *et al.* Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 2002;106:2067-2072.
 13. Kretowski A, Mironczuk K, Karpinska A, *et al.* Interleukin 18 promoter polymorphisms in type 1 diabetes. *Diabetes* 2002;51:3347-3349.
 14. Kretowski A, Kinalska I. Serum levels of interleukin-18 – a potential marker of cardiovascular death – could be determined by genetic predisposition. *Circulation* 2003;107:e206.