

Serum and aqueous humor concentrations of interleukin-27 in diabetic retinopathy patients

Maha E. Houssem · Mona Abo Bakr El-Hussiny · Amr El-Kannishy · Dalia Sabry · Rasha El Mahdy · Mohamed E. Shaker

Received: 11 February 2017 / Accepted: 15 July 2017 / Published online: 24 July 2017
© Springer Science+Business Media B.V. 2017

Abstract

Purpose Interleukin (IL)-27 has been reported to possess anti- and proinflammatory properties in several immune related-disorders, but its role in diabetic retinopathy is still elusive. Here, we aimed to (i) evaluate IL-27 concentrations in serum and aqueous humor of diabetic patients with or without retinopathy and (ii) test whether IL-27 is correlated with some risk factors of diabetic retinopathy.

Methods The study comprised 60 diabetic patients with and without retinopathy along with 20 healthy

controls. Serum and aqueous humor concentrations of IL-27 were assessed by ELISA.

Results The mean of IL-27 concentration in aqueous humor in patients with diabetic retinopathy (6.7 ± 2.7 ng/L) was significantly elevated in comparison with either diabetic patients without retinopathy (4.6 ± 0.5 ng/L) or healthy control group (4.1 ± 0.8 ng/L). Besides, IL-27 concentration in aqueous humor was positively correlated with serum glucose, lipid profile and glycated hemoglobin (HbA1c).

Conclusions Based on this study, IL-27 is implicated in the pathogenesis of diabetic retinopathy and positively correlates with the disorder progression.

Keywords Diabetic retinopathy · IL-27 · Glycated hemoglobin · Cholesterol · Triglycerides

M. E. Houssem
Department of Biochemistry, Faculty of Pharmacy,
Damanhour University, Damanhour, Egypt

M. A. B. El-Hussiny
Department of Clinical Pathology, Faculty of Medicine,
Mansoura University, Mansoura 35516, Egypt

A. El-Kannishy · D. Sabry
Department of Ophthalmology, Faculty of Medicine,
Mansoura University, Mansoura 35516, Egypt

R. El Mahdy
Department of Medical Microbiology and Immunology,
Faculty of Medicine, Mansoura University,
Mansoura 35516, Egypt

M. E. Shaker (✉)
Department of Pharmacology and Toxicology, Faculty of
Pharmacy, Mansoura University, Mansoura 35516, Egypt
e-mail: mshaker2222@yahoo.com

Introduction

Diabetes mellitus is associated with numerous complications, which can be life-threatening, if not controlled. Of these complications, diabetic retinopathy has gained much interest as a severe progressive ocular pathology and the leading cause of blindness among aged patients [1]. Recent advances have shown that inflammation arising during diabetes has a key role in diabetic retinopathy development and progression [2]. Diabetes biochemical alterations, including

increased advanced glycation end-products formation, protein kinase C activity and flux of polyols and hexosamines, induce an increase in oxidative stress and inflammatory responses [3]. The latter response is characterized by overproduction of diverse proinflammatory and pro-angiogenic mediators, including (but not limited to) intercellular adhesion molecule (ICAM)-1, tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-6 and vascular endothelial growth factor (VEGF) [4]. The increased production of the inflammatory mediators causes degeneration of retinal capillaries via recruiting leukocytes to endothelium, leading to an increase in vascular permeability and thrombosis probability [5]. The recruited leukocytes eventually produce more proinflammatory and pro-angiogenic factors, leading to further infiltration of leukocytes in the retina, alteration of the blood retinal barrier and neovascularization to overcome the ischemic insult caused by vascular occlusion [6].

IL-12 family members, IL-23, IL-27 and IL-35, have emerged as a critical mediators of T helper (Th)1 and Th17 cell populations responses [7]. In particular, the heterodimeric cytokine IL-27 consists of the Epstein-Barr virus-induced gene 3 and IL-27p28, which comprises a receptor composed of gp130 and the IL-27Ra that transduces signals via activation of JAK/STAT (Janus kinase/signal transducer and activator of transcription) and MAPKs (mitogen-activated protein kinases) [8]. IL-27 was initially recognized as a proinflammatory cytokine, because of its enhancement of production of interferon (IFN)- γ and proliferation in CD4+ T cells [9]. However, several studies have reported the negative regulatory effects of IL-27 on Th1, Th2, and Th17 cell responses and elucidated the various functions mediated by this cytokine, including its capability to stimulate IL-10 production and block T cell production of IL-2, which directly suppresses Th2 and Th17 cell activities [10, 11]. Thus, modulating IL-27 clinically might be beneficial in managing inflammatory conditions associated with aberrant immune response.

On this background, the present study aimed to estimate IL-27 concentration in serum and aqueous humor samples of diabetic patients with or without retinopathy and to determine the degree of correlation between its concentration and other risk factors of diabetes, such as serum glucose, cholesterol, triglycerides levels and glycated hemoglobin (HbA1c).

Subjects and methods

The aqueous humor and blood samples were collected from healthy (control group) and diabetic patients. All diabetic patients were at least 10 years of type 2 diabetes mellitus. The participants were selected from Mansoura Ophthalmic Center through 2015–2016. They were categorized into 4 groups: 20 control non-diabetics, 20 diabetic patients without retinopathy, 20 diabetic patients with non-proliferative diabetic retinopathy and 20 diabetic patients with proliferative diabetic retinopathy. Non-proliferative diabetic retinopathy cases were having microaneurysm, retinal hemorrhage, edema and exudates, and in case of appearance of neovascularization either at the optic disk or elsewhere by fluorescein angiography, this was assigned as proliferative diabetic retinopathy. An informed consent was obtained from each patient subsequent to approval of the study by the ethical committee (Faculty of Medicine, Mansoura University). All employed procedures were concordant with the ethical regulations of the committee related with human experimentation (institutional and national) and Helsinki Declaration in 1975 and revise in 2008.

Exclusion criteria

Patients with uncontrolled hypertension or other systemic disease rather than diabetes mellitus, patients with any eye disease rather than cataract and/or diabetic retinopathy and patients with previous treatment for diabetic retinopathy, e.g., laser or intraocular injection was excluded from the study. Cases with dense cataract that prevent fundus examination were also excluded. All patients underwent general examination and laboratory tests to exclude systemic diseases. Complete ocular examination was performed including visual acuity testing slit lamp biomicroscopic examination of anterior segment, intraocular pressure measurement and posterior segment assessment using contact and non-contact lens. Fluorescein angiography was performed for all diabetic patients.

Sample collection

The aqueous humor samples (around 0.1 mL) were drained from the anterior chamber using insulin syringe before initiation of incisions of cataract surgery in groups 1, 2, 3 or prior anti-VEGF injection

in group 4. Blood samples were then collected from each participant in the study after 12–14 h of overnight fasting. Blood samples were divided into two portions. The first portion (1 mL) of whole blood portion was collected in tubes containing EDTA and was used for detection of HbA1c. The second portion was centrifuged at 3000 *g* for 10 min for separation of serum. The resultant serum was further divided into two other portions. One of them was used for immediate determination of triglycerides, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and glucose, whereas the other was kept at $-70\text{ }^{\circ}\text{C}$ until IL-27 assay.

Biochemical analysis

The concentration of triglycerides and total cholesterol in serum were estimated as previously described [12, 13] by a kit purchased from Spinreact (Spain; with a detection limit of 0.6 mg/dL). Also, HDL concentration in serum was estimated as previously mentioned [14] by a kit purchased from Spinreact (Spain; with a detection limit of 1.57 mg/dL). LDL concentration in serum was determined from the equation of Friedewald et al. [15]. Glucose concentration in serum was determined by the method of Trinder [16] using a kit purchased from Spinreact (Spain). HbA1c levels were estimated in the whole EDTA blood samples by the ion exchange resin method of Trivelli et al. [17] using kits purchased from Stanbio (USA). Serum and aqueous humor concentrations of IL-27 were measured by enzyme-linked immunosorbent assay (ELISA) kit purchased from Glory Science Co. Ltd (TX, USA) with a detection limit of 1.6 ng/L.

Statistical analysis

Continuous variables are expressed as mean \pm standard deviation (SD). Data were first examined for normality and equality of distribution before performing any analysis. One-way ANOVA test was used for comparing the continuous data among the groups. The correlations between the data with the serum and aqueous IL-27 levels were performed using the Pearson correlation co-efficient test. Statistical analysis was performed by SPSS 20.0 statistical software package (Chicago, USA). The level of significance was set at $P < 0.05$.

Results

The contributing subjects were categorized into four groups as follows: 20 diabetic patients with proliferative diabetic retinopathy, 20 diabetic patients with non-proliferative diabetic retinopathy, 20 diabetic patients without retinopathy and 20 sex- and age-matched control non-diabetic subjects (Table 1).

One-way ANOVA indicated that there were significant differences between the investigated groups in serum glucose ($F = 11.483$, $P < 0.001$) and HbA1c ($F = 7.436$, $P < 0.001$) concentrations. One-way ANOVA showed also that there were significant differences between the groups in serum cholesterol ($F = 7.720$, $P < 0.001$), triglycerides ($F = 17.079$, $P < 0.001$), HDL ($F = 6.217$, $P < 0.001$) and LDL ($F = 6.094$, $P < 0.001$) concentrations (Table 1).

The mean of aqueous humor IL-27 concentration was elevated in proliferative diabetic retinopathy (6.1 ± 2 ng/L) and non-proliferative diabetic retinopathy (6.7 ± 2.7 ng/L) patients, but not diabetic patients without retinopathy (4.6 ± 0.5 ng/L), as compared to the control group (4.1 ± 0.8 ng/L). This elevation was found to be statistically significant by one-way ANOVA ($F = 9.669$, $P < 0.001$) (Table 1).

The mean of serum IL-27 concentration was also elevated in proliferative diabetic retinopathy (4.6 ± 3.9 ng/L) and non-proliferative diabetic retinopathy (4.6 ± 4.4 ng/L) patients, but not diabetic patients without retinopathy (2.4 ± 0.8 ng/L), as compared to the control group (2.9 ± 1.2 ng/L). This elevation was also found to be statistically significant by one-way ANOVA ($F = 3.020$, $P = 0.035$) (Table 1).

Correlation analysis among proliferative diabetic retinopathy patients demonstrated significant correlations between aqueous humor IL-27 concentration and serum glucose ($r = 0.499$, $P = 0.025$), cholesterol ($r = 0.453$, $P = 0.045$), triglycerides ($r = 0.461$, $P = 0.041$) and HbA1c ($r = 0.573$, $P = 0.008$) concentrations (Table 2).

Correlation analysis among non-proliferative diabetic retinopathy patients showed also significant correlations between aqueous humor IL-27 concentration and serum glucose ($r = 0.511$, $P = 0.021$), cholesterol ($r = 0.568$, $P = 0.009$), triglycerides ($r = 0.495$, $P = 0.026$) and HbA1c ($r = 0.501$, $P = 0.025$) concentrations (Table 3).

Table 1 Demographic and laboratory characteristics of the studied groups

	Proliferative retinopathy <i>N</i> = 20 Mean ± SD	Non-proliferative retinopathy <i>N</i> = 20 Mean ± SD	Diabetic without retinopathy <i>N</i> = 20 Mean ± SD	Controls <i>N</i> = 20 Mean ± SD	ANOVA	
					<i>F</i>	<i>p</i>
Clinical characteristics						
Age (years)	65.2 ± 5	63.1 ± 4.2	62.8 ± 5	63.5 ± 4	1.131	0.342
Duration (years)	15.8 ± 3.1	14.5 ± 3.3	14.8 ± 3.2		0.956	0.391
Blood sugar						
Glucose (mg/dL)	165.1 ± 100.1**	189 ± 76.3***	188.3 ± 44***	82 ± 10.3	11.483	<0.001
HbA1c (%)	6.7 ± 1.6*	7.5 ± 1.7***	6.8 ± 1.8*	5.3 ± 0.8	7.436	<0.001
Lipid profile						
Cholesterol (mg/dL)	156.4 ± 43.6**	164.3 ± 49.9***	153.6 ± 44.6**	108.1 ± 17.6	7.720	<0.001
Triglycerides (mg/dL)	152.4 ± 43.3***	170.9 ± 70***	149.8 ± 24***	80.2 ± 9.6	17.079	<0.001
HDL (mg/dL)	44.2 ± 10.8	38.6 ± 6**	38.8 ± 6.2**	47.9 ± 8.3	6.217	<0.001
LDL (mg/dL)	82.3 ± 43.4**	85.5 ± 44.8**	84.7 ± 45**	40.8 ± 16.4	6.094	<0.001
IL-27						
Serum IL-27 (ng/L)	4.6 ± 3.9	4.6 ± 4.4	2.9 ± 0.8	2.4 ± 1.2	3.020	0.035
Aqueous humor IL-27 (ng/L)	6.1 ± 2**	6.7 ± 2.7***	4.6 ± 0.5	4.1 ± 0.8	9.669	<0.001

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *HbA1C* glycated hemoglobin

Statistical significances are * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ from the control group

Table 2 The correlation between IL-27 concentrations in serum and aqueous humor and patients' characteristics in the proliferative retinopathy group

	Serum IL-27		Aqueous IL-27	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age (years)	0.119	0.616	−0.157	0.509
Duration (years)	0.292	0.212	0.002	0.992
Glucose (mg/dL)	−0.288	0.218	0.499	0.025
HbA1c (%)	−0.111	0.642	0.573	0.008
Cholesterol (mg/dL)	−0.324	0.163	0.453	0.045
Triglycerides (mg/dL)	−0.361	0.118	0.461	0.041
HDL (mg/dL)	0.087	0.716	−0.261	0.267
LDL (mg/dL)	−0.273	0.244	0.419	0.066
Serum IL-27 (ng/L)			−0.305	0.191
Aqueous humor IL-27 (ng/L)	−0.305	0.191		

Correlation analysis among diabetic patients without retinopathy demonstrated significant correlations between aqueous humor IL-27 concentration and serum HbA1C ($r = 0.489$, $P = 0.028$) and IL-27 ($r = 0.457$, $P = 0.043$) concentrations (Table 4).

Discussion

Diabetic retinopathy is a vision-threatening complication of systemic diabetes mellitus. In the present

study, we assessed the levels of circulating and aqueous humor IL-27 and investigated their correlation with some conventional risk factors for retinopathy. To the best of our knowledge, studies that assess IL-27 concentration, especially in the aqueous humor of diabetic retinopathy patients, are still scarce.

The results revealed an elevation of IL-27 concentration in aqueous humor of diabetic retinopathy patients (either proliferative or non-proliferative) in comparison with diabetic patients without retinopathy or healthy individuals. Thus, diabetic retinopathy is

Table 3 The correlation between IL-27 concentrations in serum and aqueous humor and patients' characteristics in the non-proliferative retinopathy group

	Serum IL-27		Aqueous IL-27	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age (years)	0.367	0.111	−0.148	0.534
Duration (years)	0.350	0.130	−0.125	0.599
Glucose (mg/dL)	0.025	0.917	0.511	0.021
HbA1c (%)	0.090	0.705	0.501	0.025
Cholesterol (mg/dL)	−0.217	0.357	0.568	0.009
Triglycerides (mg/dL)	−0.311	0.182	0.495	0.026
HDL (mg/dL)	0.218	0.355	0.149	0.531
LDL (mg/dL)	−0.152	0.522	0.214	0.365
Serum IL-27 (ng/L)			−0.299	0.200
Aqueous humor IL-27 (ng/L)	−0.299	0.200		

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *HbA1C* glycated hemoglobin

Table 4 The correlation between IL-27 concentrations in serum and aqueous humor and diabetic patients' characteristics without retinopathy

	Serum IL-27		Aqueous IL-27	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Age (years)	−0.284	0.225	−0.134	0.574
Duration (years)	−0.131	0.583	−0.118	0.620
Glucose (mg/dL)	−0.478	0.033	−0.024	0.919
HbA1c (%)	0.086	0.719	0.489	0.028
Cholesterol (mg/dL)	−0.055	0.816	0.093	0.696
Triglycerides (mg/dL)	−0.233	0.324	0.135	0.571
HDL (mg/dL)	0.210	0.373	−0.061	0.797
LDL (mg/dL)	−0.060	0.801	0.089	0.710
Serum IL-27 (ng/L)			0.457	0.043
Aqueous humor IL-27 (ng/L)	0.457	0.043		

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *HbA1C* glycated hemoglobin

correlated with an enhanced local synthesis and secretion of IL-27. Lee et al. [18] suggested that the retinal cells limited the intraocular inflammation via increasing the production of IL-27 and IL-10. The anti-inflammatory effect of IL-27 in retinal cells is mediated through different mechanisms. IL-27 promotes the expansion of a population of IL-10 secreting T cells [19]. Increased production of IL-10 upregulates suppressor of cytokine signaling 3 that negatively regulates proinflammatory cytokine activities, thereby making retinal cells tolerant to stimulation of these cytokines in chronic intraocular inflammatory diseases [20]. IL-27 can also suppress TH17 development and effector T cells expansion via targeting several signal transduction proteins [21–23].

Antunica et al. [24] did not find any significant increase in serum concentration of IL-12, which is the most influential stimulator of Th1 differentiation and

IFN- γ production in diabetic subjects with retinopathy. However, Zorena et al. [25] found a marked elevation in IL-12 concentration in serum of diabetic retinopathy patients, compared with healthy subjects. The discordance in the data of both studies can be attributed to the variable rates of patients' microangiopathy. Our results indicated that serum IL-27 concentration was elevated in diabetic retinopathy patients. Despite its retinal anti-inflammatory properties, IL-27 may have proinflammatory potential systemically. IL-27 can stimulate the differentiation of naïve CD4 T cells into Th1 and which then produce IFN- γ by the action of IL-12 [9, 26, 27].

Our results revealed a significant correlation between IL-27 in aqueous humor and some conventional risk factors for retinopathy, such as serum glucose levels, glycemic control as assessed by HbA1C, cholesterol and triglycerides levels. Our data

are in harmony with those of Schram et al. [28] who demonstrated that the proinflammatory cytokines IL-6 and TNF- α were in a good correlation with some risk factors for retinopathy. On the other hand, our data were in disagreement with the studies conducted by Winkler et al. [29] and Antunica et al. [24], who found that IL-12 did not correlate with risk factors of diabetic retinopathy. According to our data, there were correlations between serum and aqueous humor IL-27 concentrations and risk factor parameters only in diabetic subjects without retinopathy. Based on this finding, diabetic patients without retinopathy possess an unaltered hemato-ocular barrier devoid of abnormalities in local synthesis of IL-27.

In conclusion, this study showed a marked elevation of both serum and aqueous humor IL-27 concentrations in diabetic retinopathy patients. Also, the aqueous humor concentration of IL-27 correlated with some diabetic retinopathy risk factors like serum glucose and HbA1C concentrations. More studies are still required to fully investigate the biological effects of IL-27 on the characteristic changes associated with retinopathy and phenotyping of the patient groups, especially in relation to aspects of diabetic control and drug-treatment regimes.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest to disclose.

References

- Cheung N, Mitchell P, Wong TY (2010) Diabetic retinopathy. *Lancet* 376(9735):124–136
- Adamis A (2002) Is diabetic retinopathy an inflammatory disease? *Br J Ophthalmol* 86(4):363–365
- Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* 414(6865):813–820
- Goldberg RB (2009) Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. *J Clin Endocrinol Metab* 94(9):3171–3182
- dell’Omo R, Semeraro F, Bamonte G, Cifariello F, Romano MR, Costagliola C (2013) Vitreous mediators in retinal hypoxic diseases. *Mediat Inflamm* 2013:935301
- Noda K, Nakao S, Ishida S, Ishibashi T (2012) Leukocyte adhesion molecules in diabetic retinopathy. *J Ophthalmol* 2012:279037
- Yoshida H, Nakaya M, Miyazaki Y (2009) Interleukin 27: a double-edged sword for offense and defense. *J Leukoc Biol* 86(6):1295–1303
- Kastelein RA, Hunter CA, Cua DJ (2007) Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* 25:221–242
- Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, Hibbert L, Churakova T, Travis M, Vaisberg E (2002) IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4+ T cells. *Immunity* 16(6):779–790
- Hunter CA, Kastelein R (2012) Interleukin-27: balancing protective and pathological immunity. *Immunity* 37(6):960–969
- Liu H, Rohowsky-Kochan C (2011) Interleukin-27-mediated suppression of human Th17 cells is associated with activation of STAT1 and suppressor of cytokine signaling protein 1. *J Interferon Cytokine Res* 31(5):459–469
- Bucolo G, David H (1973) Quantitative determination of serum triglycerides by use of enzymes. *Clin Chem* 19(5):476–482
- Meiattini F, Prencipe L, Bardelli F, Giannini G, Tarli P (1978) The 4-hydroxybenzoate/4-aminophenazone chromogenic system. *Clin Chem* 24(12):2161–2165
- Burtis CA, Ashwood ER, Bruns DE (1999) Tietz. In: *Textbook of Clinical Chemistry*, 3rd edn. WB Saunders, Philadelphia, PA
- Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18(6):499–502
- Trinder P (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol* 22(2):158–161
- Trivelli LA, Ranney HM, Lai H-T (1971) Hemoglobin components in patients with diabetes mellitus. *N Engl J Med* 284(7):353–357
- Lee YS, Amadi-Obi A, Yu CR, Egwuagu CE (2011) Retinal cells suppress intraocular inflammation (uveitis) through production of interleukin-27 and interleukin-10. *Immunology* 132(4):492–502
- Awasthi A, Carrier Y, Peron JP, Bettelli E, Kamanaka M, Flavell RA, Kuchroo VK, Oukka M, Weiner HL (2007) A dominant function for interleukin 27 in generating interleukin 10-producing anti-inflammatory T cells. *Nat Immunol* 8(12):1380–1389
- Liu X, Mameza MG, Lee YS, Eseonu CI, Yu C-R, Derwent JJK, Egwuagu CE (2008) Suppressors of cytokine-signaling proteins induce insulin resistance in the retina and promote survival of retinal cells. *Diabetes* 57(6):1651–1658
- Villarino AV, Stumhofer JS, Saris CJ, Kastelein RA, de Sauvage FJ, Hunter CA (2006) IL-27 limits IL-2 production during Th1 differentiation. *J Immunol* 176(1):237–247
- Diveu C, McGeachy MJ, Boniface K, Stumhofer JS, Sathe M, Joyce-Shaikh B, Chen Y, Tato CM, McClanahan TK, de Waal Malefyt R (2009) IL-27 blocks ROR γ expression to inhibit lineage commitment of Th17 cells. *J Immunol* 182(9):5748–5756
- Hirahara K, Ghoreschi K, Yang X-P, Takahashi H, Laurence A, Vahedi G, Sciumè G, Hall AOH, Dupont CD, Francisco LM (2012) Interleukin-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1. *Immunity* 36(6):1017–1030

24. Antunica AG, Karaman K, Znaor L, Sapunar A, Buško V, Puzović V (2012) IL-12 concentrations in the aqueous humor and serum of diabetic retinopathy patients. *Graefes Arch Clin Exp Ophthalmol* 250(6):815–821
25. Zorena K, Mysliwska J, Mysliwiec M, Balcerska A, Lipowski P, Raczynska K (2008) Interleukin-12 and tumour necrosis factor-alpha equilibrium is a prerequisite for clinical course free from late complications in children with type 1 diabetes mellitus. *Scand J Immunol* 67(2):204–208
26. Takeda A, Hamano S, Yamanaka A, Hanada T, Ishibashi T, Mak TW, Yoshimura A, Yoshida H (2003) Cutting edge: role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. *J Immunol* 170(10):4886–4890
27. Owaki T, Asakawa M, Fukai F, Mizuguchi J, Yoshimoto T (2006) IL-27 induces Th1 differentiation via p38 MAPK/T-bet-and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways. *J Immunol* 177(11):7579–7587
28. Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, Stehouwer CD (2003) Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes. *Diabetes Care* 26(7):2165–2173
29. Winkler G, Dworak O, Salamon F, Salamon D, Speer G, Cseh K (1998) Increased interleukin-12 plasma concentrations in both, insulin-dependent and non-insulin-dependent diabetes mellitus. *Diabetologia* 41(4):488–488